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PETROLOGY OF THE MAXVILLE LIMESTONE FROM PARTS OF MUSKINGUM AND PERRY COUNTIES, OHIO[1]

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Scope of the investigation

The Maxville limestone from parts of Muskingum and Perry Counties, Ohio, was studied in order to determine its mineral composition and texture and to interpret its depositional and postdepositional history in this area.

Previous work on the Maxville limestone has been mainly field mapping, paleontological study, and chemical analysis.

The field work for this paper was done in the autumn of 1951 and in the spring of 1952. Stratigraphic sections of the Maxville limestone were measured at five localities (fig. 1) in western Perry County and southwestern Muskingum County. One section was measured from a core which was drilled east of Philo, in Wayne Township, Muskingum County. Most of the samples were collected at 1-foot intervals at the time the sections were measured. Specimens from a few thin units were combined so as to have one sample for each foot of the beds that were measured. In units more than 1 ft. thick, specimens were collected from each foot and combined to form one sample for the unit. For a unit over 5 or 6 ft. thick, the upper half was designated "a" and the lower half "b". A sample then was collected from each half of the unit. Sample localities are as follows:

Locality 1. At type locality, abandoned quarry, center sec. 17, T. 14 N., R. 16 W., Monday Creek Township, Perry County.

Locality 2. Abandoned quarry, SE¼ NW¼, sec. 25, T. 17 N., R. 17 W., Reading Township, Perry County.

Locality 3. At entrance to mine operated by Pittsburgh Plate Glass Company near Fultonham, SW¼ NW¼, sec. 20, T. 15 N., R. 14 W., Newton Township, Muskingum County.

Locality 3a. In a large quarry of the Pittsburgh Plate Glass Company near Fultonham, SE¼ SE¼, sec. 18, T. 15 N., R. 14 W., Newton Township, Muskingum County.

Locality 4. A drilled core near Philo, SE¼ NE¼, sec. 29, T. 13 N., R. 12 W., Wayne Township, Muskingum County.

Locality 5. West end of Kent Run gorge, NW¼ NW¼, sec. 2, T. 17 N., R. 15 W., Newton Township, Muskingum County.

Petrographic analyses were made at The Ohio State University during the winter and spring of 1952. A total of 116 insoluble residues were prepared and examined under a stereoscopic binocular microscope. Ten thin sections were studied, and 15 specimens were etched and/or stained.

[1]A paper dealing with this subject was submitted to the Graduate School in partial fulfillment of the requirements for the degree, Master of Science in the Department of Geology at The Ohio State University, June, 1952.

Stratigraphy of the Maxville limestone

Definition and distribution. Andrews (1870, p. 83) named the Maxville limestone for exposures near Maxville, Monday Creek Township, Perry County, Ohio. He described the Maxville as a limestone formation which "always rests upon the top of the Logan sandstone group" with disconformity and is overlain disconformably by the Pottsville formation.

The Maxville limestone crops out in isolated patches between southeastern Scioto County (Morse 1910, p. 81) and Hopewell Township, Muskingum County (Stout 1921, p. 37). The limestone is well-exposed in the Fultonham area, in the banks of Jonathan Creek and Kent Run in western Muskingum County, and in the banks of Little Monday and Little Rush Creeks and their tributaries in Perry County (fig. 1).

The Maxville limestone is quarried and mined extensively for use in cement manufacture in the Fultonham area. In southeastern Ohio it formerly was used for road metal, railroad ballast, agricultural lime, furnace flux, lime for mortar, and building stone.

Lithology and thickness. The lower 10 to 27 ft. of the Maxville limestone in southeastern Ohio consist of fine- to medium-grained light gray-tan, light-gray, or light blue-gray limestone, dolomitic limestone, or dolomite. The lower part is dense, massive, arenaceous, somewhat clayey, and sparsely fossiliferous. The upper 22 to 43 ft. consist of fine- to medium-grained light gray-tan to blue-gray dense limestone, which is fossiliferous and well-bedded.

Both the upper and lower parts of the Maxville limestone are exposed in the Fultonham area. Here, near the top of the lower part, is an unusual wavy contact between two dolomitic limestone units. The crests in the wavy contact are about 3 to 5 ft. apart.

At the type locality at Maxville (No. 1 of figure 1 and 2), at Kent Run, and at the abandoned quarry in Reading Township, 10 to 15 ft. of the lower part of the Maxville limestone are present, but the upper part is absent. The lower part, at these localities, consists of very fine- to fine-grained tan-gray to light blue-gray massive limestone, dolomitic limestone, or dolomite. At Maxville, a layer of calcareous chert 10.3 in. thick composes the lower-most unit of the limestone. This chert probably corresponds to units B3 and C5 in the sections described by Morse (1910, p. 74–75).

A core which was drilled in Wayne Township, Muskingum County (No. 4 of figure 1 and 2), penetrated 70 ft. of the Maxville limestone and included both the upper and lower part of the formation. The upper part consists of fine-grained . . . light-gray to light tan-gray . . . dense . . . sparsely fossiliferous limestone, interbedded with calcareous . . . silty green-gray, tan-gray, or dark-gray shale. The lower 15 ft. 2 in. of the upper part of the formation are sparsely oolitic. The lower part of the formation is 27 ft. thick, and it is fine-grained gray-tan massive dense limestone, dolomitic limestone, and dolomite. The lower 9 feet of the Maxville limestone are composed mainly of limestone breccia and at least two zones of calcareous oolites. Morse (1910, p. 60) described a unit which contains minute calcareous concretions from the Maxville in the Kent Run area. This unit possibly corresponds, in part at least, either to unit 4, 6, or 7 of the cored section, each of which contains calcareous oolites.

Forty-two feet of the limestone are exposed in the large quarry in the Fultonham area. At this locality only the upper contact is exposed.

Age and correlation. The Maxville limestone is the youngest formation of the Mississippian system exposed in Ohio. Morse (1910, p. 108) stated that fossils from the Maxville, identified by Ulrich and others, indicate a Ste. Genevieve age. Weller (1948, p. 160) identified trilobites from the limestone as Ste. Genevieve in age. The Maxville limestone thus can be considered as belonging to the Meramecian series.

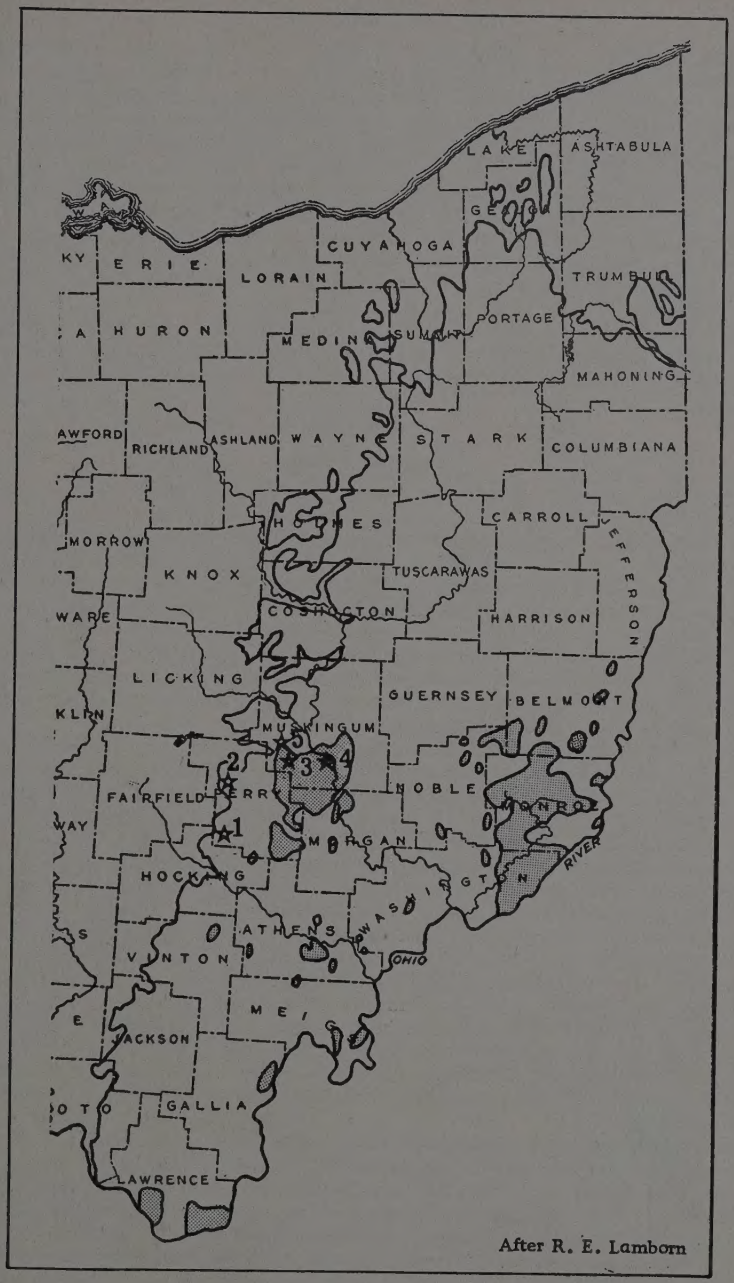

FIGURE 1. Map of Eastern Ohio showing the line of outcrop of the Mississippian–Pennsylvanian contact, areas of occurrence of the Maxville limestone below drainage, and localities of samples taken.

Prior to 1948, the Maxville was believed to be equivalent in age to the Greenbrier limestone in West Virginia, Virginia, southern Pennsylvania, and Maryland; to the Ste. Genevieve limestone in eastern Missouri, southern Illinois, Indiana, Iowa, Kentucky, Tennessee, northern Alabama, and northern Mississippi; and to the Bayport limestone in Michigan (Morse 1910, p. 111; Wilmarth 1937, p. 129, 886–887, 1324–1325, 1873–1876). In 1948, the Mississippian Subcommittee of the Geological Society of America published a chart in which some significant differences in correlations of the Maxville limestone are shown. The Maxville limestone is shown in large part as equivalent in age to the Big Lime subsurface formation in southwestern Pennsylvania and north-central West Virginia, and to the top of the lower part of the Greenbrier series in southern Randolph County, West Virginia.

The writer believes that the textures of the Maxville limestone and relative percentages of insoluble minerals in this limestone are similar to those in part of the Greenbrier limestone described by Rittenhouse (1949, p. 1704–1730).

PETROLOGY

Insoluble residues

Procedure. A total of 116 samples of the Maxville limestone were reduced to small chips and digested in a 50 percent solution of hydrochloric acid. About one-fourth of the samples were heated to the boiling point of the acid solution in order to quicken digestion; the rest of the samples were digested at room temperature. After digestion had been completed, the acid was decanted, and the residues were washed. A few of the residues were crushed and rewashed in order to separate shaly particles from the coarse residue. After each washing had been completed, the contents of the beakers were stirred, and the coarse residue was allowed to settle. The fine residues were not studied. The coarse residue was dried and weighed in the beaker. The percentages of the coarse residues are shown on graphs (fig. 2).

Each coarse residue was studied under a binocular microscope to determine the mineral content, the size and shape of the grains, and the relative percentage of each mineral present. Thirty-five residues were mounted in Canada balsam and examined under a petrographic microscope in order to identify tourmaline, zircon, and feldspar, and in order to see inclusions in the detrital grains of quartz.

Mineral composition of the coarse residue. The insoluble minerals present in the Maxville limestone are detrital and secondary quartz, pyrite, magnetite, hematite, albite, orthoclase, microcline, tourmaline, and zircon. Inclusions of semi-opaque needles of rutile are present in some of the detrital grains of quartz. Banded chalcedony, a variety of quartz, is present in only one of the residues.

Detrital grains of quartz are abundant in the lower part of the Maxville limestone, but are rare in the upper part. Secondary quartz is abundant in the upper part but rare in the lower part. Although pyrite, magnetite, and hematite are rare in the Maxville limestone in most localities, they are abundant in the Fultonham area. Orthoclase and microcline are rare; tourmaline and zircon are present in small amounts in the lower part, and are rare or absent in most localities in the upper part.

Detrital grains of quartz average 81 percent of the total coarse residue in the lower part of the Maxville limestone and 13 percent in the upper part. These quartz grains range from 0.03 mm. to 1.00 mm. in diameter and average 0.11 mm. to 0.24 mm. Thus some of the grains are silt size, some are medium sand size, and most are fine sand size. The quartz grains are transparent or frosted, angular, subangular, or subrounded. Frosted subangular to subrounded grains are dominant. Secondary quartz has been deposited on many of the detrital grains and can be seen with a binocular microscope. A few of the grains that show secondary

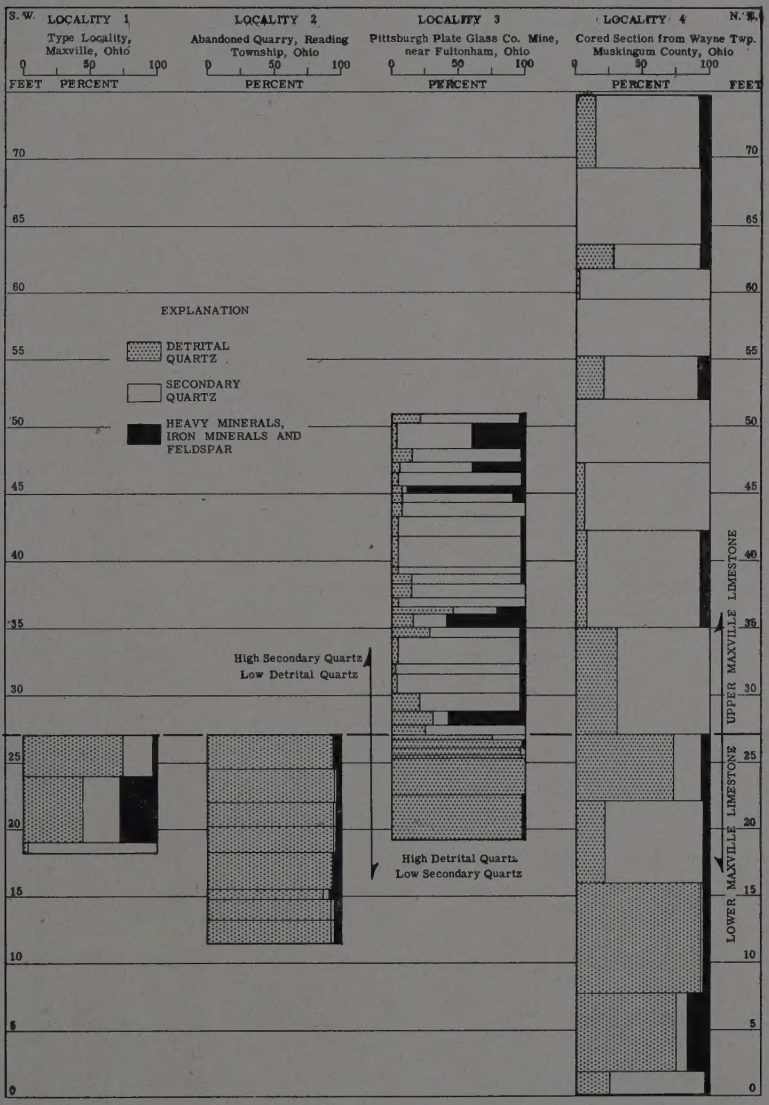

FIGURE 2. Graphs of coarse insoluble residues, showing the percentage relationships of detrital and secondary quartz, and heavy minerals.

growth are doubly terminated short prisms. The outline of the original subrounded detrital grains, with an overgrowth of quartz which forms well-developed crystal faces, can be seen with a petrographic microscope. Small carbonate rhombs are included in some of the secondary quartz that has been deposited on the detrital grains. Inclusions of semi-opaque needles of rutile and short prismatic grains of tourmaline are present in a few detrital grains of quartz.

Aggregates of secondary quartz average 56 percent of the coarse residue in the upper part of the Maxville limestone and 12 percent in the lower part. Most of the aggregates are snow white; some are gray to tan. The aggregates are rod-shaped, in fossil fragments, in shapes suggesting fossil fragments, and in irregular shapes. The rod-shaped aggregates range from 0.04 mm. to 3.28 mm. in length, and the fossil fragments have a maximum diameter of 3.87 mm. At Maxville, the type locality, an insoluble residue from the lowermost unit in the section is 99 percent secondary quartz. This quartz is in clear to milky angular grains and banded aggregate grains of chalcedony. A mass of orange quartz is present in the center of a few of the banded grains.

Irregular aggregates and grains of brassy-yellow pyrite average 7 percent of the coarse residues from the Maxville limestone; in the Fultonham area the pyrite averages 10 percent. The aggregates and grains range from 0.08 mm. to 0.30 mm. in diameter. Most of the pyrite aggregates are granular and show poorly to well-developed minute pyritohedral and cubic crystal faces; octahedra are rare. Pyrite in the shape of rods also is rare. Aggregates of pyrite mixed with secondary quartz are present in a few of the residues.

Magnetite octahedra and aggregates of magnetite and hematite average 8 percent of the coarse residues in the Maxville limestone; in the large quarry near Fultonham, however, these minerals average 20 percent. The aggregates and octahedra range from 0.13 mm. to 0.40 mm. in diameter. Magnetite and hematite commonly are in the same aggregates.

Feldspar is present in the upper and lower parts of the Maxville limestone in amounts ranging up to 6 percent, and averaging 0.2 percent, of the coarse residues. Euhedral grains of albite and orthoclase show no signs of abrasion and therefore are authigenic. Two subrounded grains of microcline and microperthite were identified; these are detrital.

Tourmaline and zircon are rare or absent in the residues from the upper part of the Maxville limestone, but they are up to 3 percent in many of the residues from the lower part. Most of the tourmaline grains are dark blue-green or amber in plain light under the petrographic microscope. Most of the tourmaline grains are rounded, some are elongate, and a few are equant with sharp edges. The tourmaline ranges from 0.05 mm. to 0.33 mm. in diameter, and therefore is of very fine to fine sand size. Most of the zircon grains are pink or red and oval or subrounded. The size range of the zircon is the same as that of the tourmaline. Under the petrographic microscope a few of the zircon grains appear highly fractured.

Thin sections and etched and stained specimens

General statement. Seven thin sections were prepared from the lower and three from the upper part of the Maxville limestone from the localities shown in figure 1. A total of 15 specimens from the lower and upper parts of the limestone were cut in two and ground flat with carborundum powder on a glass plate. Each of the cut specimens was etched by immersion in dilute hydrochloric acid for 30 to 90 sec. All specimens but one were stained, after they were etched, by immersion in a boiling solution of malachite green for 3 to 4 min.

Textural varieties. A study of the thin sections and the etched specimens shows five textural varieties; four are from the lower part of the Maxville limestone and one is from the upper part.

One textural variety from the lower part of the Maxville is dolomitic limestone breccia from the lower part of the cored section. The fragments are composed of dolomite rhombs about 0.01 mm. in diameter and some calcite, and the matrix consists of slightly coarser dolomite and calcite. Detrital grains of quartz are present in the rock unit in amounts up to 3 percent, and are present largely in the matrix. Small carbonate rhombs are included in secondary quartz that has been deposited on the detrital grains of quartz. In the matrix, aggregates of secondary quartz which have replaced calcite are rare.

A second textural variety is present in a few of the units from the lower part of the Maxville, and it shows calcareous oolites ranging from 0.17 mm. to 1.00 mm. in diameter. Etching reveals the concentric structure of the round, subround, oval, or elongate oolites. A few of the oolites have a core of detrital quartz rimmed by concentric layers of very fine-grained calcite. Most of the oolites contain cores of colorless transparent calcite, which is coarser grained than that in the concentric layers. The matrix is colorless calcite which composes about 15 percent of the oolitic limestone. Irregular patches of coarser calcite and some of chalcedony are scattered unevenly throughout the matrix. Fibrous calcite, which may be fossil material, is present in variable but small amounts.

A third textural variety, from the lower part of the Maxville, is shown by very fine- to fine-grained limestone containing scattered patches of colorless calcite which are coarser grained than the matrix. Some of this coarser calcite occurs in fossil fragments. Fibrous calcite is rare. Detrital grains of quartz are present in the rock unit in amounts up to 2 percent. Small carbonate rhombs are included in secondary quartz that was deposited on these detrital grains.

A fourth textural variety, from the lower part of the Maxville, is shown by very fine-grained dolomitic limestone, of which slightly less than half is composed of closely spaced subhedral to euhedral dolomite rhombs averaging 0.01 mm. in diameter. The other half is fine-grained calcite. Scattered patches of calcite coarser grained than the matrix are rare. Some of this coarser calcite occurs in fossil fragments. A trace of fibrous calcite, which may be fossil material, is present. Detrital grains of quartz are present in the rock unit in amounts up to 25 percent and averaging 5 percent. A few spherulites of chalcedony that show radial extinction are present. Rounded or oval grains of tourmaline and zircon are rare. This fourth texture was seen only in thin section.

The most typical texture of the upper part of the Maxville limestone shows abundant fossil fragments and shapes suggesting fossil fragments. This texture is clearly shown by the etched surfaces. Most of the upper part of the Maxville consists of fine-grained calcite; the calcite in the fossil fragments is transparent and coarser grained than that in the rest of the rock. Fossil fragments rarely are rimmed by a thin layer of secondary quartz which stands above the rest of the etched surface.

Diagenesis and secondary replacement

General statement. Insufficient facts are available to determine the sequence of formation of the diagenetic and secondary minerals present in the Maxville limestone. Dolomite probably is diagenetic. Secondary minerals are pyrite, quartz, chalcedony, albite, orthoclase, magnetite, and hematite.

Dolomite. The Maxville changes from dolomite and dolomitic limestone to limestone within a distance of a few miles, as is shown by variable amounts of dolomite in the same parts of the formation from different measured sections. In the Fultonham area and in the cored section the dolomitic limestone contains cavities and is more porous than the overlying limestone.

Magnesium-bearing waters may have circulated through the lower part of the limestone and thus deposited dolomite which replaced calcite. If the dolomite replaced calcite before the induration of the Maxville was complete, the replacement occurred during the diagenetic stage of rock formation.

Secondary quartz, pyrite, and feldspar. Secondary quartz is abundant only in the upper part of the Maxville limestone. Most of the quartz occurs as replacements in rod-shaped fragments, in shapes suggesting fossils, and in fossils.

The chert bed, in the section at Maxville, the type locality, consists largely of clear to milky angular microcrystalline quartz. None of the quartz shows signs of abrasion; it is believed to be secondary. The bed probably was porous limestone in which the calcite in large part was replaced by quartz.

As shown by a study of the thin sections, inclusions of carbonate rhombs which may be dolomite are present in some of the aggregates of secondary quartz. This quartz may have replaced calcite; the dolomite, which may not have been so readily replaced, was entrapped in the quartz.

Secondary quartz, pyrite, and feldspar (albite and orthoclase) may have formed slightly below the surface of deposition throughout Maxville time. Another possibility is that in post-Maxville time these minerals may have been precipitated from circulating ground-water. Finally, the minerals may have developed both during and after deposition of the Maxville. The correct hypothesis cannot be determined at this time.

The secondary feldspar consists of albite and orthoclase crystals. These crystals show no evidence of rounding and are believed to be authigenic.

Magnetite and hematite. Insoluble residues from the sections from Kent Run, from the abandoned quarry in Reading Township, and from the mine near Fultonham average 20 percent of magnetite and hematite. Most of the magnetite is present in aggregates with irregular shapes like the secondary quartz, thus suggesting that the magnetite also is secondary. Excellent magnetite octahedra rarely are present. The octahedra show no evidence of rounding and are believed to be authigenic. Spiroff (1938, p. 818) synthesized magnetite octahedra, and he described an occurrence in Michigan of magnetite which probably was deposited from meteoric solutions. Brown (1943, p. 137–148) summarizes 10 occurrences of low temperature authigenic magnetite. Two of these occurrences are in the Grenville limestone (pre-Cambrian) of Ontario, Canada, and Balmat, New York.

It is believed that the magnetite in the Maxville limestone probably was formed in post-Maxville time by circulating ground-water Friedman (1954, p. 101).

This mineral is present throughout the section from the large quarry, where its abundance suggests that there may have been a local concentration of iron salts in the sediments overlying the Maxville at that place. Many of the aggregates of insoluble iron material from the Maxville contain both red hematite and black magnetite. Small amounts of magnetite are present in some hematite aggregates as shown by the attraction of some of the aggregates by a magnet. Thus it seems likely that the hematite in the Maxville was formed by oxidation of the authigenic magnetite.

Secondary growth on detrital grains of quartz. Most of the detrital grains of quartz in the material studied show secondary growth. This growth has resulted in some grains that show doubly terminated short prisms. The grains with secondary growth contain cores of detrital quartz which can be seen clearly in the mounted residues under a petrographic microscope. Under crossed nicols some of the cores show one or more color rings, whereas the secondary quartz growth is gray or white of the first order. The color difference occurs because the cores are thicker than the secondary growth.

As seen in the thin sections, secondary growth on detrital grains of quartz is suggested by inclusions of small carbonate rhombs.

CONCLUSIONS

The insoluble minerals present in the Maxville limestone are, in order of decreasing abundance, secondary quartz including chalcedony; detrital quartz; secondary pyrite, magnetite, and hematite; detrital tourmaline, zircon, microcline, and microperthite; and secondary feldspar (albite and orthoclase).

The upper or high-calcium part of the Maxville limestone may be differentiated from the lower or dolomitic part by a comparison of the relative percentages of the detrital and secondary quartz present in the insoluble residues. Detrital grains of quartz average 81 percent of the total coarse residue from the samples studied from the lower part and 13 percent from the upper part. Aggregates of secondary quartz average 12 percent of the coarse residue from the lower part and 56 percent from the upper part.

Chemical analyses of the limestone have been made, but they may not be useful in distinguishing the upper part from the lower part in places where the magnesium content is not sufficiently high to be diagnostic of the lower part. In this case, a study of the insoluble residues may be useful.

The cored section from Wayne Township, Muskingum County, contains about 30 ft. of high calcium limestone between the depths of 330 and 360 ft. The lower 15 ft. are in part silicious. Insoluble residues show that these 30 ft. range from 91 to 95 percent carbonate. If the magnesium content does not exceed 5 percent, this part of the formation could be mined for use in cement manufacture.

Magnetite which is authigenic occurs in the Maxville limestone. An examination of the geological literature of the United States indicates that the occurrence of authigenic magnetite in a Mississippian limestone previously has not been reported.

ACKNOWLEDGMENTS

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Elements of Zoology. *Tracy I. Storer* and *Robert L. Usinger*. McGraw-Hill Book Co., Inc., New York. xii+552 pp. $5.50.

Elements of Zoology is an abridgement of Storer's highly successful and well-known General Zoology. The reduction of one-third has been accomplished primarily in Part II which treats the animal kingdom. Part I on animal biology remains essentially the same size but the material is rearranged and rewritten. Elements of Zoology is designed for a one semester course. The content favors substantial instruction in classical zoology. We predict this text, like its parent, will find wide use.

CARL VENARD.

GEOLOGIC AND TECTONIC FEATURES OF THE COYOTE MOUNTAINS, ARIZONA

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The granitic rocks of the Coyote Mountains in south central Arizona exhibit a well defined planar structure which strikes to the northwest and dips steeply to the southwest. The range was examined in an attempt to cast some light on the character of the granitic rocks in this part of the state and to determine the configuration of their internal structures. Previous work in the area by McGee (1897), Bryan (1922, 1923) and Darton (1925) has been largely confined to descriptions of topography and analyses of sedimentary processes in arid regions. This paper proposes to describe briefly the rocks and tectonic fabric of this hitherto relatively unknown part of southern Arizona.

LOCATION AND DESCRIPTION

The Coyote Mountains are located 30 miles west of Tucson, at the northern extremity of the Baboquivari chain. The Baboquivari Mountains trend northward from the Mexican border essentially following the 111° 40′ meridian for a distance of more than 30 miles and are one of a group of sub-parallel ridges in southern Arizona.

The Coyote Mountains mass is roughly triangular in plan (fig. 1). Steep, rugged slopes are common and much of the area is virtually inaccessable. The highest point is Coyote Peak which lies in the northwestern part of the mapped area. Relief is approximately 3300 ft. and plan area exceeds 22 sq. mi.

Folded volcanic rocks of the Roskruge Mountains lie to the northeast of the Coyote Mountains, and granitic rocks belonging to the Quinlan group are found to the west. Both the Roskruge and Quinlan ranges are separated from the Coyote Mountains by major faults which are reflected in the topography of the area by steep, eroded scarps and low passes.

THE ROCKS

General statement. Metamorphic and igneous rocks of several types are exposed in the Coyote Mountains, and a brief description of each type is included below, although a thorough discussion of the petrology and petrography of these rocks is not within the scope of this paper. Suffice it to say that many of the varieties mentioned, when examined in thin section, reveal highly irregular mineral borders, mineral overgrowths, and numerous tiny inclusions, all of which are indicative of crystalloblastic growth and perhaps of additive metamorphism. Shearing, crushing, and evidence of recrystallization are to be seen in some of the specimens (especially the alaskites), indicating that the rocks have undergone one or more phases of dynamic metamorphism. Although an origin by granitization for some of these rocks has not been proven, the petrographic features just mentioned as well as the evidence afforded by the arrangement of internal structures indicates that the process should not be ruled out.

Sedimentary rocks. Red shales, conglomerates, and siltstones, tentatively correlated with the Cretaceous (?) Recreation red beds (Brown, 1939) crop out along the Ajo Road fault zone and probably underlie portions of the Roskruge Mountain extrusives farther north. Within the Coyote Mountains, the only direct evidence of a former sedimentary rock is the presence of marble and quartzite masses which are now surrounded by diorite.

Monzonite. Rocks of undetermined origin, which have compositions ranging from granite through monzonite to quartz diorite, occupy most of the southern

and eastern portions of the Coyote Mountains. All these rocks are shown as monzonite on figure 1. Inclusions of hand sized clots of biotite-rich schistose rock are common throughout most of the monzonite and related rock types. The orientation of these clots, as well as the orientation of biotite flakes, hornblende crystals, and feldspar porphyroblasts, contribute to the planar structure conspicuous in these rocks.

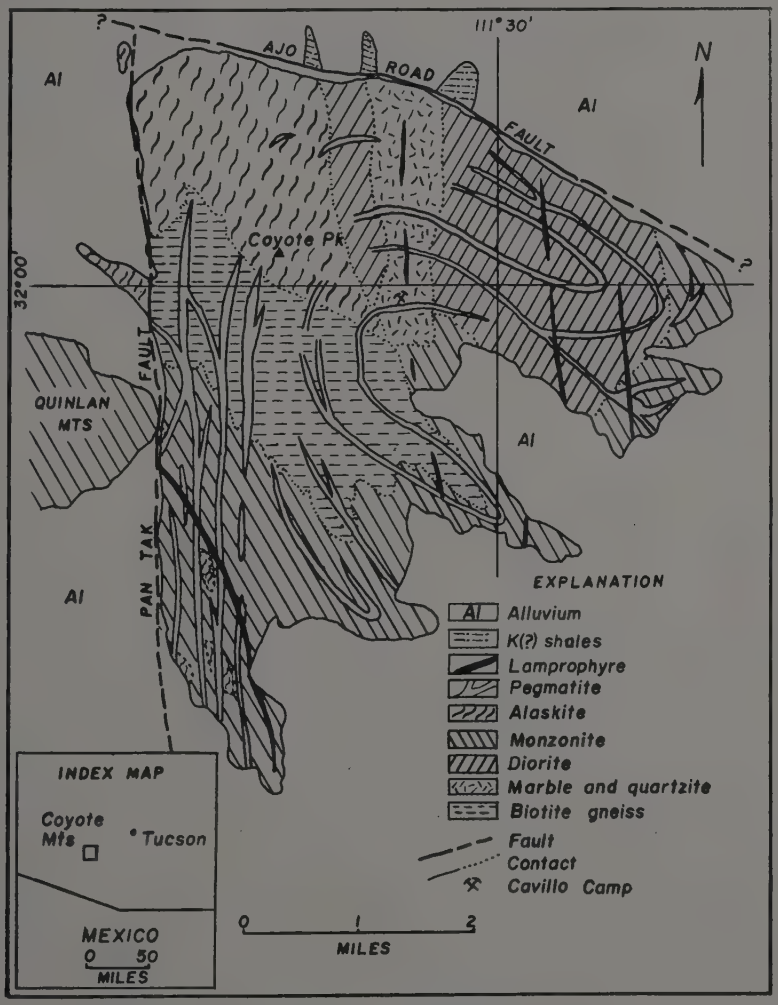

FIGURE 1. Geologic map of the Coyote Mountains, Arizona.

Pegmatite. Pegmatite is widespread throughout most of the mountain mass. The occurrences can be grouped into three classes: (1) tabular, dike-like bodies dipping 20°–30° east to southeast, some of which are as much as 80 ft. thick, (2) irregular, anastomosing veinlets as much as one foot thick, which probably repre-

sent stringers from the larger masses, and (3) diffuse masses of pegmatite which literally "soak" the enclosing rock. The mineralogy of the pegmatite is relatively simple—quartz, orthoclase and plagioclase with minor biotite, muscovite and red garnet. Large crystals of perthitic orthoclase are common. The northwest strike of planar elements is retained in blocks of biotite gneiss and monzonite that

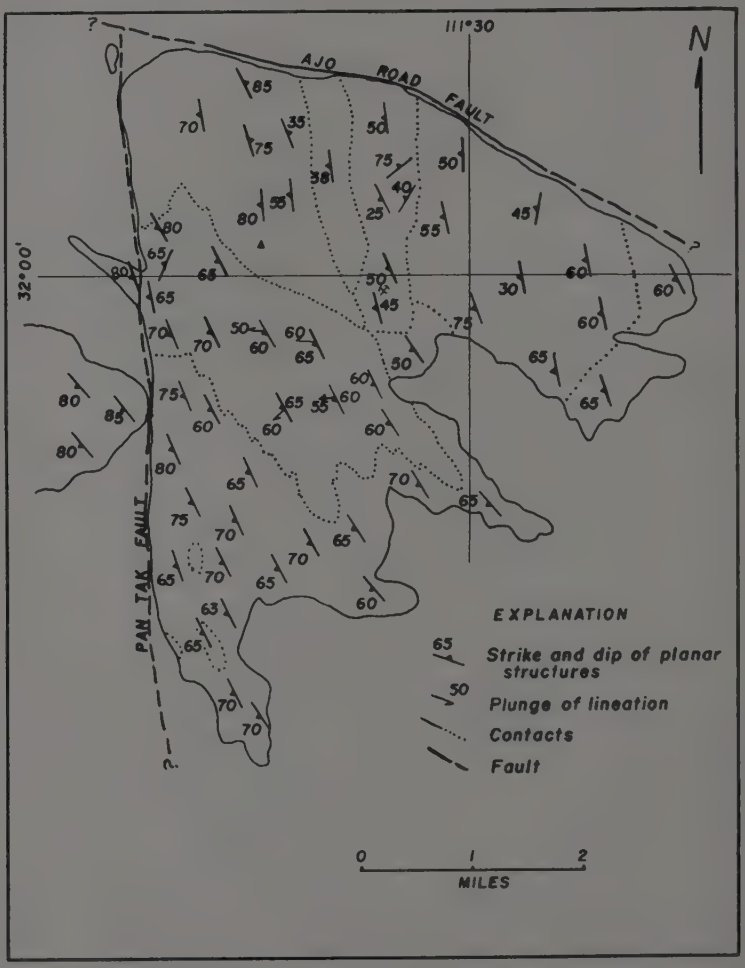

FIGURE 2. Tectonic map of the Coyote Mountains, Arizona.

have been included between the larger pegmatite bodies, and the blocks do not appear to have been displaced or rotated. In some places the planar elements found in the enclosing rocks can be traced into the pegmatite. In figure 1 the pegmatite outcrops are generalized and the unusual pattern is a reflection of the effect of rugged topography on shallow dipping layers.

Biotite gneiss. A northwest striking band of biotite gneiss approximately one and one-half miles wide is found in the central portion of the Coyote Mountains mass. The rock is a typical fine-grained biotite-orthoclase-quartz gneiss, which in part grades into a flaky biotite schist. The contact between the gneiss and the surrounding rocks is sharp, but extremely irregular. Near the alaskite the gneiss is bleached and somewhat altered, and contacts are less distinct. The largest of the tabular pegmatite bodies cut the biotite gneiss.

Alaskite. Rocks mapped as alaskite and consisting mainly of quartz and orthoclase crop out in the northwestern part of the Coyote Mountains and in scattered locations to the south (fig. 1). The largest mass of alaskite is strongly sheared, and thin sections of the rock show extreme rounding of feldspars as well as recrystallization of quartz with attendant flamboyant extinction patterns. Where-

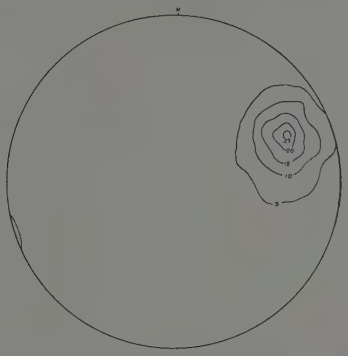

FIGURE 3. Equal-area projection of the perpendicular poles of planar structures, Coyote Mountains, Arizona. 300 readings, 5% contours. Plotted on the lower hemisphere.

ever biotite is found, a crude planar orientation can be seen and the attitude of the planes of shearing is revealed. Minor, flat-lying shear planes are found near the Ajo Road fault. The outcrops of alaskite found in the southern portion of the range do not show this shearing.

Diorite. Rocks ranging in composition from diorite to gabbro, with some small masses of hornblendite are located in the northern portion of the mapped area. Planar structures in the diorite are formed by preferentially oriented biotite flakes and hornblende crystals within the rock mass. The diorite encloses the elongate block of metamorphosed sediments described below, and both are cut by lamprophyre dikes and pegmatite masses, the largest of which are shown on figure 1.

Marble and Quartzite. Banded, impure marbles and quartzites are located in the central part of the Coyote Mountains. Where bedding planes are recognized in these rocks, they seem to parallel the trend of the planar elements found in the surrounding rocks, although the erratic attitudes of these bedding planes in the center part of the band suggests folding and/or rotation of small fault blocks. Garnet, diopside and epidote (clinozoisite?) are present in moderate amounts. Attitudes of planar structures in the marble and quartzite have not been included in the equal-area plot (fig. 3).

Copper-tungsten deposits of minor consequence are found in the Cavillo Camp mining area. The ore minerals occur as fracture fillings and replacements in the metamorphosed sediments.

Dike Rocks. In addition to the rocks described above, numerous small dikes of lamprophyre and rhyolite are found throughout the region. Generally, the dikes strike north to northwest, but dip more steeply than the planar structures in the enclosing rocks. These dikes cut across all other rocks and are thought to represent the latest phase of igneous activity in the area.

INTERNAL STRUCTURES

Internal structures in the rocks of the Coyote Mountains are manifest in oriented hand-sized clots of a biotite-rich schistose rock and in oriented flakes of biotite, crystals of hornblende and porphyroblasts of feldspar. Generally these elements indicate a well developed planar structure in all the major rock types, with the exception of the pegmatite and the dikes. Lineation in the plane of foliation is noted in the biotite gneiss and rarely in the alaskite, but recognizable linear structures appear to be absent in the other rocks. Planar structures in the alaskite are less noticeable owing to the virtual absence of dark clots and biotite flakes.

The tectonic map (fig. 2) indicates a persistent trend of planar structures within the Coyote Mountains. An equal-area plot of the perpendicular poles of planar structures is shown in figure 3, and the contoured distribution serves to emphasize the persistence of a northwest trend. An average strike and dip reading is approximately N 24° W, 64° SW.

Folding can be recognized in the vicinity of the major faults that bound the mountain range on two sides. Localized buckling of the biotite gneiss near the Pan Tak fault on the west side of the range has produced folds with steeply plunging axes. Most of these folds apparently are the result of drag along the Pan Tak fault, which intersects the planar elements of the gneiss at an acute angle. The presence of these folds, coupled with the gradual swing of other planar elements into parallelism with the fault and the dragging effect observed on the alaskite dike west of the fault, indicate a strike-slip component to the faulting, with the Coyote Mountains block moving relatively southward.

Owing to the lack of exposures and general absence of well developed planar structures, the nature of the movement along the Ajo Road fault has not been determined. Presence of Cretaceous (?) sediments to the north indicates that the mountain block has been thrust upwards, but no concrete evidence of strike-slip movement was observed. Planar structures in the diorite cut by the Ajo Road fault imply movement to the northeast while those in the alaskite suggest a movement in the opposite direction.

The topography along the southeast face of the Coyote Mountains further reflects the trend of planar elements. A glance at figure 1 shows that the canyons and ridges along the southeastern extremity of the range roughly parallel the strike of the planar structures found in the rest of the mountain mass.

Joints. Joints are not a conspicuous feature in most parts of the Coyote Mountains. The attitude of widely spaced joint planes has controlled erosion on the north face of Coyote Peak, and the appearance, as noted by Bryan (1923), is that of a large tilted block of bedded sediments. Elsewhere the joint sets are less spectacular. Generally the joints strike to the northeast, cutting the trend of planar elements at almost right angles. Joints are best developed in the finer grained rocks, and the joint planes usually die out as they pass into the coarser grained rocks. Exfoliation of the granitic rock has produced massive domes in the vicinity of the Cavillo Camp mining area.

AGE OF THE ROCKS

The geologic age of the Coyote Mountains rocks and their structure has not been determined. The following items, however, may have a bearing on the solution of this problem.

A comparison with pre-Cambrian trends elsewhere in southern Arizona reveals that the Coyote Mountains planar structures strike almost at right angles to the trend of normal pre-Cambrian structures. Several possibilities for such an occurrence present themselves, among which are: (1) the Coyote Mountains represent a broad fold in the pre-Cambrian terrain, (2) the Coyote Mountains block has been rotated into its present position, or (3) the structures, and possibly the rocks are not pre-Cambrian.

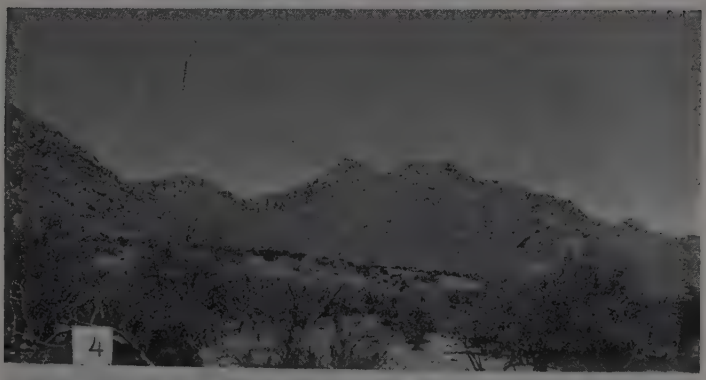

FIGURE 4. General view of the Coyote Mountains. The white patches are pegmatite dikes.

FIGURE 5. Swarm of small dark clots in the monzonite. View is taken normal to the strike of the clots. Marks on hammer handle are one inch long.

FIGURE 6. Close-up of small clot in coarse grained monzonite. The small white streak is a pagmatite veinlet.

The general uniformity of orientation of planar elements over the whole Coyote Mountains block, as well as in parts of the range to the west (fig. 2), weakens the first argument. The final test of this premise would require the examination of the structures in the rocks farther west and south to determine whether or not a fold existed. From the information at hand, which is based on a study of the Coyote and part of the Quinlan mountains, it does not appear that these rocks represent a flexure in a pre-Cambrian terrain.

That some rotation has occurred, especially along the Pan Tak Fault, is inferred from the fact that planar structures west of the fault strike 5 to 10 degrees more to the northwest and have steeper dips than those to the east. However, rotation on a scale sufficient to cause an 80° to 90° change in strike direction has not been recognized.

The suggestion that the majority of the rocks, or at least their internal structures (if the two are not of the same age), are younger than pre-Cambrian is based on the presence of possible Paleozoic metamorphosed sediments in the area, and on the suggestions made by other workers who have conducted studies in this part of the state. Although no fossils have been found, the metamorphosed limestone beds previously mentioned bear a strong likeness to limestones of probable Paleozoic age in the Tucson and Sierrita Mountains to the east (Brown, 1939, Ransome, 1921). Since the Coyote Mountains marbles and associated metasediments appear to be a block partially engulfed by dioritic rocks of undetermined origin, these surrounding rocks may be younger than pre-Cambrian and possibly younger than Paleozoic. Furthermore, the recognition at Bisbee (Butler and Wilson, 1938) and at Ajo (Gilluly, 1937) of Mesozoic tectonic activity seems to link the Coyote Mountains rocks and their structures to the tectonic events of that era. Paleotectonic and paleogeologic maps of the Late Jurassic presented by Eardley (1951) include the area in the vicinity of the Coyote Mountains in the Chico Shuni orogenic belt, the tectonic activity in which is provisionally correlated with that of the Nevadan Revolution.

Summarizing these last few points, it seems that an age younger than pre-Cambrian should be considered for the Coyote Mountains rocks and their internal structures because of:

1. Nonconformity of structural trends in the Coyote Mountains with normal northeast pre-Cambrian trends found in other parts of southern Arizona.
2. Presence of metamorphosed sediments which may be of Paleozoic age.
3. Known orogenic activity capable of producing the Coyote Mountains rocks and their structures, which activity is generally assigned to the Mesozoic era.

ACKNOWLEDGMENTS

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RADIAL GROWTH OF BEECH AND SOIL MOISTURE IN A CENTRAL OHIO FOREST DURING THE GROWING SEASON OF 1952.[1]

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In ecent years there has been considerable interest and research on micro-environments in central Ohio (Wolfe *et al.*, 1949). Christy (1952) and DeSelm (1952) measured the temperatures and carbon dioxide gradients respectively in a beech-maple forest. During 1952 Gilbert (1953 and unpublished data) measured the rainfall interception and the soil moisture at Blacklick Woods, one of the forest parks near Columbus, Ohio. The author believed that additional measurements on the growth of trees during the same period might help to relate some of these factors to the development of the forest community.

Beech, *Fagus grandifolia* Ehrh., was chosen as the study tree, for it is a dominant member of the beech-maple association and extends into the adjacent swamp forest to the edges of the depressions which in spring are woodland pools.

REVIEW OF LITERATURE

Interest in tree growth is by no means a recent development. According to Glock (1941) the literature of the nineteenth century contains many references to various aspects of tree growth. The earliest studies dealt mainly with the nature and significance of growth rings. Later interest also included meristematic and cambial growth and their relationship to heredity and environment. Friesner (1943) discusses some of the pertinent literature on tree growth under three subdivisions: elongation, seasonal diameter growth, and annual ring studies of past years.

There is considerable literature dealing with the interrelationships between tree growth and environment. Adams (1935) reviews much of this and discusses many of the factors. He points out that variations in radial growth of trees may be due to hereditary differences, physiological differences due to conditions and age of the individual, and differences in the environment.

Several other workers, among whom are Glock (1941) and Robbins (1921), have also recognized differences in growth due to heredity and physiological conditions. MacDougal (1924) has demonstrated from growth studies that the seasonal growth period of an individual tree varies with age as well as with different physiological conditions. He reports that the growth period in *Pinus radiata* was longest in young trees while wider differences in growth were exhibited by older trees.

Others, Lyon (1940), Glock (1950), and Lodewick (1930), report that many of these variations in growth of individual trees are overshadowed by environmental influences in a particular site. Schulman (1941) states that the relative importance of any one of the environmental factors operating on tree growth varies from region to region and site to site.

Friesner (1941, 1942), Lodewick (1928), and MacDougal (1936) have compared seasonal climatic conditions to cambial activity and radial increase in growth. The varying effects of environmental factors throughout the season are mentioned by Lundengardh (1931). In temperate latitudes he divides the year into four ecological climatic periods; (1) the growth period of spring, (2) the assimilation period including summer and early autumn, (3) the fruit formation period, and (4) the winter of after-ripening period. He states that growth is primarily determined by the climate of spring and photosynthesis by the climate of mid-summer and autumn.

[1]Department publication number 565.

MacDougal (1938) shows that in general the time of initiation of cambial growth in *Pinus radiata* depends largely upon temperature, and time of cessation varies from year to year depending largely upon time of exhaustion of the water supply. Friesner (1941) in his work on beech states, "In the earlier part of the season, when water supply is most likely to be adequate, temperature is more likely to become the limiting factor, whereas in the later part of the season, when temperature is adequate, available water and evaporation become the limiting factors. . . . As temperature increases the demand for water to supply evaporation losses increases also and thus makes available water a still more potent limiting factor."

Reineke (1932) introduced a dial-gauge dendrometer for precise measurement of radial growth, and more recently an improved dendrometer was described by Daubenmire (1945) and is now widely used.

LOCATION AND DESCRIPTION OF THE STUDY AREA

The forest in which this study was conducted is a relatively undisturbed area called Blacklick Woods and is located 10 miles east of Columbus, Ohio, and a mile SSW of Reynoldsburg, Ohio. It is now one of the several Columbus Metropolitan Parks. It lies in the extreme northwest corner of Fairfield County, its western border lying on the Franklin-Fairfield County line and its northern part extending into Franklin County. It is located at about 82° 49′ west latitude and 39° 56′ north longitude.

The area is on the gently undulating late Wisconsin till plain, with elevations varying from 870 to 895 ft. Three soil types of the Alexandria catena are present. These are Marengo silty clay loam in the depressions where water accumulates nearly every spring, Bennington silt loam in the surrounding level to gently sloping areas, and Cardington silt loam on the crests of the gentle rises. The differences in relief between these soil types may be only a few feet, yet the plant communities at the two extremes are distinctly different.

The dominant species occurring in the depressions are: *Ulmus rubra*,[2] *Ulmus americana*, *Fraxinus americana*, *Acer rubrum* and *Acer saccharinum*. The dominant species on the higher sites are: *Fagus grandifolia*, *Acer saccharum*, *Ulmus rubra* and *Ulmus americana*. Less prominent species in the study area are: *Tilia americana*, *Carya ovata*, *Carya cardiformis*, *Juglans nigra*, *Prunus serotina*, *Nyssa sylvatica*, *Quercus bicolor*, *Quercus palustris*, *Acer negundo*, *Ostrya virginiana*, *Asimina triloba*, and *Crataegus* spp. A dense understory of spice bush, *Lindera benzoin*, characterizes both communities except in the areas of the woodland pools and in the most mature and well drained sites.

Beech occurs in different proportions between the depressions and the highest sites in the gradiation from swamp forest (Sampson 1930) to beech-maple. An analysis of this transition in the forest studied was made by sampling areas of similar relief by means of walking transects through each area. Care was taken to record every tree within arm's length and over 5 ft. high, and not to include any one individual more than once. All trees were tallied to the nearest odd inch dbh. The sampling areas occupy roughly three sites: near depressions (1.5 to 3.0 ft. higher than the bottom of neighboring depressions), on gentle rises (3 or more feet above the depressions), and on the highest sites (on top of knolls or along crests of rises). The total relief in the sampling area was no more than seven feet. Table 1 illustrates some of the differences between these communities.

Near the depressions elm is dominant constituting 25 percent of the community and 46 percent of the basal area with beech the next important species. However, the beeches are often small trees which are gnarled and decayed in the center. On the gentle rises elm becomes secondary to beech which constitutes 38 percent of the community and 76 percent of the basal area in this site. On the highest

[2]The nomenclature is that of *Gray's Manual of Botany* (Fernald 1950).

sites beech becomes less important as sugar maple increases in number and size in the beech-maple association.

METHODS

During the spring of 1952 three beech trees were selected from these three different habitats in the forest. Radial increase of the trunks was measured by the dendrometer method described by Daubenmire (1945) and discussed by Brown *et al.* (1947). Three 2-inch screws were inserted 1.5 in. into the tree about 5 ft. above the ground. By placing a dendrometer upon these, the distance from the heads of the screws to the bark could be measured. A small area of the bark was covered with shellac where the spindle of the instrument touched the tree. Measurements were made on three radii in each tree at intervals of several days from April 17 to November 15, between 12:00 noon and 4:00 pm. EST. Soil moisture readings were taken by Gilbert (unpublished data) near the three sites

TABLE 1

Vegetational analysis of several sites in Blacklick Woods

Species	Near Depressions		On Gentle Rises		On Highest Sites	
	Trees over five feet high	Basal area	Trees over five feet high	Basal area	Trees over five feet high	Basal area
Acer saccharum	5%	—	27%	5%	26%	22%
Fagus grandifolia	14%	31%	38%	76%	23%	62%
Ulmus rubra } *Ulmus americana*	25%	46%	14%	19%	18%	16%
Acer rubrum } *Acer saccharinum*	12%	9%	0	0	2%	—
Fraxinus americana	10%	4%	2%	—	2%	—
Other species	34%	10%	19%	—	19%	—

TABLE 2

Soil moisture constants for the Colman units in three sites at Blacklick Woods
(percent moisture)

	Depth of Unit	Horizon	Moist. Equiv.	15 atm.	P.W.P.	Lowest Record
Highest Site	7.0 inches	A_2	29.5	6.6	9.4	6.5
	22.5 inches	B_{22}	30.0	15.3	15.6	15.5
Gentle Rise	5.5 inches	A_2	29.5	6.6	9.4	15.5
	15.5 inches	B_{21}	32.0	17.3	17.5	20.5
In Depression	6.0 inches	A_1	44.5	19.5	22.8	26.5
	19.0 inches	Bg_2	45.0	16.0	17.5	26.5

throughout the period at several depths by means of the Colman fiberglas soil moisture units. Moisture equivalents were ascertained by centrifuging the sample at one thousand times gravity for 30 minutes. The percent moisture at 15 atmospheres was determined by the pressure membrane method. The permanent wilting percent (P.W.P.) was ascertained by allowing six-inch oat plants growing on undisturbed cores to wilt in controlled light and temperature rooms (table 2).

DESCRIPTION OF SITES

The tree near the depression is 100 ft. from the center of the depression and 1.5 ft. higher. All adjacent canopy trees are approximately 20 to 25 ft. distant and are elm (*U. americana* and *U. rubra*) and ash (*F. americanum*) except for one 20-inch beech on the up-slope side. The understory consists of scattered spice bush, herbaceous plants and tree seedlings. The soil at the base of the tree is Bennington silt loam towards the rise and Marengo silty clay loam towards the depression.

The tree on the gentle rise is 250 ft. up-slope from the tree near the depression, and it's base is 4.5 ft. higher. The slope rises about one foot beyond this tree leveling off into a gently undulating area of beech and elm. The tree is younger than the other two and had recently entered the canopy. It borders an opening in the canopy to the northeast, and within 15 ft. on this side are four other beeches 10, 9, 9, and 5 in. dbh as well as a dense understory of spice bush. But on the opposite side of the tree the forest floor is open with little or no spice bush. Neighboring trees on this side are mature canopy individuals of ash, beech, and sugar maple (*A. saccharum*), all further than 15 ft. from the tree.

The tree on the highest site is near the crest of a small knoll which rises on the average of three feet in 100 from the more level surrounding swamp forest. The area adjacent to the study tree is occupied by four large canopy trees of beech ranging from 18 to 26 in. dbh approximately 20 feet distant. About 30 ft. southeast there is a small area of open canopy and elm and spice bush understory. However, within a 25 ft. radius the forest floor is relatively open.

Unfortunately the soil moisture units in the depression were placed in a site 6 in. lower and 150 ft. from the tree studied. However, they were on the opposite side of the depression and not far from identical sites which lacked a suitable beech tree. Adjacent trees to the units were all elm, ash, and red maple (*A. rubrum*). The units were buried in the A and B horizon of Marengo silty clay loam (table 2) which is more poorly drained and has more organic matter in the A horizon than the neighboring Bennington soil. The moisture units on the gentle rise were 15 ft. west of the tree and in the area of little understory. The units were buried in the A and B horizon of Cardington silt loam. The units in the highest site were 20 ft. from the study tree and also within the area of little understory. They too were buried in the A and B horizon of Cardington silt loam.

The relationship between soil moisture, understory, and "competition" was studied by Korstian and Coile (1938). By trenching experiments they demonstrated that:

> "Survival of plants under natural forest canopies is dependent upon a complex of factors. It varies not only with climate and altitude, but very markedly with soil moisture, soil temperature, soil nutrients, light intensity, and other factors as well. Light intensity is usually subordinate in importance."
>
> "Marked changes occurred in vegetation of the trenched plots after trenching . Trenching was followed by increases in number of individuals, number of species, and general luxuriance of vegetation."
>
> "It is believed that the study clearly demonstrates that competition between individuals of the forest vegetation for soil moisture is a highly significant factor in the growth, development, and reproduction of forests in the Piedmont plateau."

The same phenomenon appears to exist in the study area at Blacklick Woods. Recognition of soil moisture as a primary effective variable makes the distribution of the spice bush understory intelligible. On the highest sites, where the canopy is closed and the soil moderately well drained, the understory is sparce. However, in the less well drained sites where there is lateral movement of soil water from the adjacent higher sites, and in the disturbed well drained sites where "competition" is not as great, spice bush and other understory species are abundant. But in the largest depressions where water stands until early summer the understory is again absent.

An estimate of the "competition" and moisture relationships in each site can be then be made as follows: The tree near the depression and the soil moisture units represent a mature swamp forest site where "competition" is maximum for that particular association. The units were in a slightly more moist site than the tree, but the moisture regimens probably did not differ greatly. The tree on the gentle rise bordered between a relatively mature beech-maple association and a large opening in the canopy. As a result, toward the opening "competition" as discussed by Korstian and Coile (1938) was lower and the understory abundant, while on the opposite side, "competition" was greater with little understory.

Since the moisture units were placed in this latter area, the readings in all probability represent a slightly dryer regimen than that for the total root system of the tree. If there is no future disturbance, this area will probably return to mature beech-maple.

The tree on the highest site was not only on a slightly better drained site but in a more mature community than the second tree, and hence "competition" was greater. As a result, the spice bush understory was lacking. Here the moisture readings should represent the approximate regimen for the study tree.

TABLE 3

Weather data for January through September, 1952, compared to means for 1900 to 1951

Recording Period	1952 Mean Temperature Degrees F. Columbus	1900–1951 Mean Temperature Degrees F.	1952 Mean Temperature at Blacklick Woods	1952 Precipitation in Columbus	1900–1951 Mean Precipitation	1952 Precipitation at Blacklick Woods
Jan.-Apr.	—	—	—	16.75 in	11.50 in	—
May	62.3°	62.2°	59.9°	3.04 in	3.20 in	3.02 in
June	77.0°	71.2°	73.5°	3.56 in	3.58 in	2.65 in
July	78.5°	75.4°	76.7°	0.96 in	3.46 in	1.59 in
August	73.9°	73.5°	73.4°	2.50 in	3.06 in	2.53 in
September	67.0°	67.2°	66.6°	1.84 in	2.49 in	2.80 in

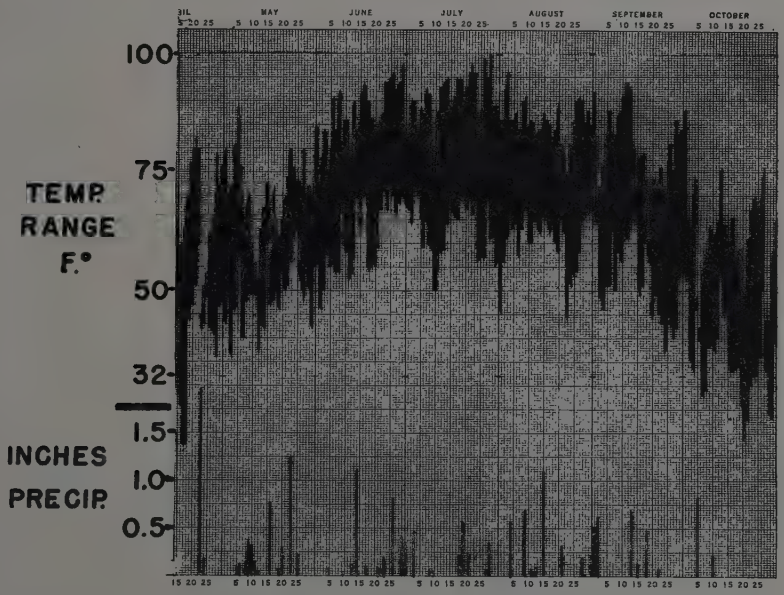

FIGURE 1. Daily Temperature Range and Precipitation During the Growing Season of 1952.

WEATHER BUREAU AND CLIMATIC DATA

Precipitation and temperature means for January through September, 1952, as calculated for the city of Columbus (U. S. Dept. of Agriculture, 1935 and U. S. Weather Bureau, 1931–1952) and at Blacklick (Gilbert 1953 and unpublished data) are given in table 3. The daily temperature range and precipitation for April 15-

October 1952 are graphed in figure 1. Unusually high precipitation during January through April resulted in abundant moisture during the spring. Assuming that the temperatures in the woods were proportionally above the average as were the city temperatures, and that the precipitation means were the same, we can conclude that the precipitation in June and July was below average and the temperature much above average in the forest. The seasonal conditions can be summarized as abundant or above average moisture in the early spring followed by below average moisture or drought conditions with high temperatures in July. This caused the spring water table to be unusually high and the July soil moisture to be unusually low.

TABLE 4

Radial increase of three beech trees from April 16 to November 1, 1952

Location	dbh	Radii	Compass Direction of Radius	Seasonal Increase in Radius
Highest Site	20.1 in	3	70°	.060 in
			330°	.047 in
			200°	.045 in
On Gentle Rise	14.1 in	3	200°	.115 in
			20°	.100 in
			330°	.083 in
Near Depression	19.6 in	3	60°	.034 in
			220°	.030 in
			310°	.025 in

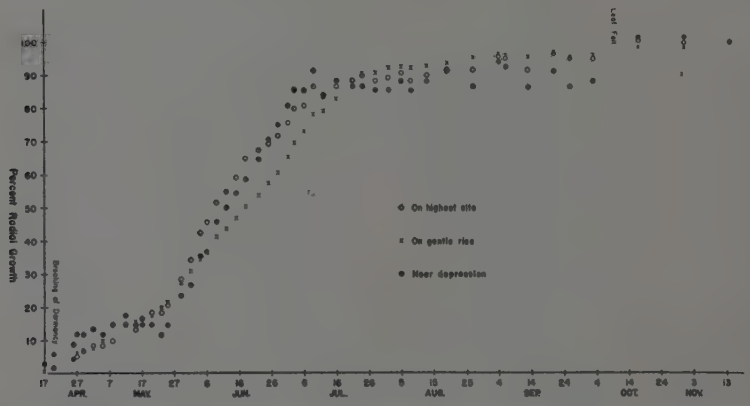

FIGURE 2. Per cent of radial growth in three beech trees on several sites during the growing season of 1952.

RESULTS

At the end of the season the growth curves for the trees were plotted and compared. In table 4 the dbh of the three trees and the radial increases for each radius is given. Even though the total increments were variable, the growth curves were proportional, so the radius with the greatest increase was chosen and the data converted to percent of total growth and plotted in figure 2. From the curves it is apparent that the three trees resembled those described for beech by Daubenmire and Deters (1947), Friesner (1941), and Jackson (1952). The breaking of dormancy occurred approximately April 20 and was subsequently

followed by radial changes. However, a continuous regular increase did not start until between May 17 to 27 which marked the beginning of the grand period of growth (Friesner 1943). The period of maximum growth continued through June and the first week in July. This was followed by cessation of growth which was not completed in one of the trees until early September.

Although some fluctuations in tree size occurred in late April and early May, the period of greatest fluctuation began in July and lasted until leaf-fall in October. After leaf-fall all the radii increased from .002 to .005 in. and remained at a relatively constant value until the end of the recording period. Since the measurements were made in the afternoon, when the water deficit due to transpiration was greatest (MacDougal 1936) it is not startling to find sizeable fluctuations in the radius. It appears (figure 2) that the fluctuations in the tree near the depression were larger than those in the other two trees. However, this is only the result of converting the data to percent growth. The tree near the depression grew the least and as a result the fluctuations became a larger percent of the total growth than in the other two trees.

TABLE 5

Deviations in three beech trees from the 7:00 AM readings during July 12, 1952
(in thousandths of inches)

Location and Site	Compass Direction of Radius	Approximate Time of Record					
		7:00 am	10:30 am	1:15 pm	2:45 pm	4:25 pm	6:30 pm
Highest Site	70°	0.0	−1.5	−2.0	−2.5	−2.5	−2.0
	330°	0.0	−1.5	−2.5	−3.5	−4.0	−3.5
	200°	0.0	−1.0	−2.5	−4.0	−3.5	−3.0
On Gentle Rise	200°	0.0	−1.5	−2.0	−2.5	−2.5	−2.0
	20°	0.0	−2.0	−2.0	−3.0	−3.0	−2.0
	330°	0.0	−2.0	−2.5	−3.0	−3.0	−2.5
Near Depression	60°	0.0	−1.5	−2.5	−3.0	−2.5	−2.5
	220°	0.0	−1.0	−2.0	−2.5	−2.0	−2.0
	310°	0.0	−1.0	−1.5	−2.0	−1.5	−1.5

As a check on the actual daily fluctuations that occur among the trees, measurements were made on all radii at several hour intervals during July 12, 1952. The day was clear with low humidity and maximum and minimum temperatures of 93° and 58° F. The relative changes in radius are included in table 5. The radial decrease is strikingly uniform among all three trees. However, the greatest shrinkage did occur in the tree on the highest site where the soil moisture at 7 and 22 in. was lowest.

It is apparent from figure 2 that the three curves show some subtle differences in shape due to differences in growth rate. For further analysis the data are graphed in figure 3 as total growth or increase in size per 5-day periods from April 25 to September 17. Below these are the corresponding graphs of the average soil moisture percentage for 5-day periods in the A and B horizons of each site. The P.W.P. is the permanent wilting percentage and the hatched area represents the percent of available water. On inspection of the top graphs the differences in the three growth curves are apparent. The tree on the gentle rise had the maximum increase in early July after which there was a gradual reduction in growth rate with only a little further increase after the first of August. However, the tree on the highest site had its maximum increase a month earlier in June, after which there was a gradual decrease in rate until July 10. No further increases occurred until August. The tree near the depression also had a small peak in growth during the same period in June followed by 30 days of relatively uniform growth. In early July there was an abrupt cessation of growth with no further increase recorded until August.

The soil moisture in the sites near these trees also differed throughout the growing season. The soil moisture on the gentle rise decreased gradually throughout the growing season in both the A and B horizon but never reached the wilting percentage. In this site it appears that moisture was ample for growth, and the tree exhibited a similar growth pattern to the beech reported by Daubenmire and Deters (1947) which grew in an arboretum in Moscow, Idaho.

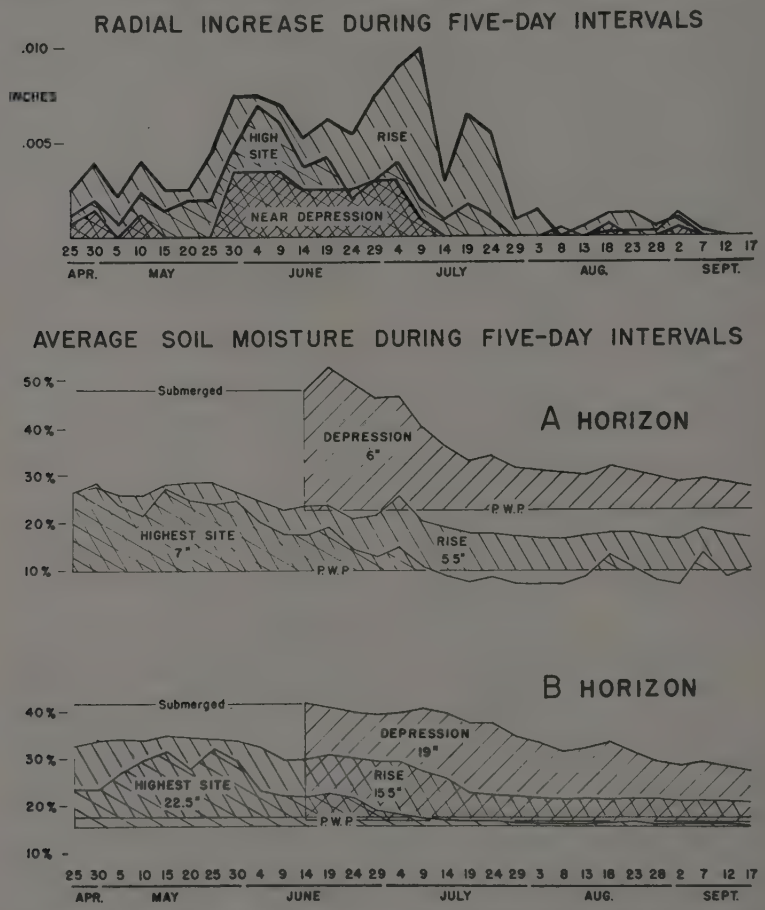
RADIAL INCREASE DURING FIVE-DAY INTERVALS
.010
INCHES
.005
HIGH SITE
RISE
NEAR DEPRESSION
25 30 5 10 15 20 25 30 4 9 14 19 24 29 4 9 14 19 24 29 3 8 13 18 23 28 2 7 12 17
APR. MAY JUNE JULY AUG. SEPT.
AVERAGE SOIL MOISTURE DURING FIVE-DAY INTERVALS
50%
40%
30%
20%
10%
Submerged
A HORIZON
DEPRESSION 6"
P.W.P
HIGHEST SITE 7"
RISE 5.5"
B HORIZON
DEPRESSION 19"
HIGHEST SITE 22.5"
RISE 15.5"
25 30 5 10 15 20 25 30 4 9 14 19 24 29 4 9 14 19 24 29 3 8 13 18 23 28 2 7 12 17
APR. MAY JUNE JULY AUG. SEPT.

FIGURE 3. Radial growth and soil moisture at three sites for five-day periods during the growing season of 1952.

The soil moisture on the highest site decreased more rapidly throughout the season, passing below the wilting percentage in the middle of July in the A horizon and approaching this value in the B horizon. It appears that the moisture in this site gradually became increasingly limiting to growth, causing a correspondingly gradual decrease in growth rate which practically stopped in July.

Soil moisture in the depression demonstrates a still different regimen. There was water in the depression until mid-June and the moisture units were submerged. By early July the soil moisture in the A horizon began to decrease rapidly. The soil moisture regimen for the tree near the depression was probably not identical, but because of its proximity to the depression, it can be inferred that it was a correspondingly wet early season followed by a similar rapid reduction in soil moisture in early July. It was at this time that radial increase abruptly stopped.

DISCUSSION

Friesner (1943) points out that "Hourly variations in diameter are recorded by the dendrograph during some parts of the vegetative season for all species studied. These daily reversible variations are entirely due to turgidity changes and set in whenever the transpiration loss exceeds the ability of the absorbing and conducting organs to supply water."

MacDougal (1938) has demonstrated a very close relation between diameter of trees and available moisture in his irrigation experiments.

Therefore, the most likely cause of the greater fluctuations (fig. 2 and 3, table 5) in the trees during the latter part of the season of 1952 is low soil moisture and high temperature and subsequently high evaporation. This is borne out by the fact that the tree on the highest site which had the least available moisture showed the greatest shrinkage (table 5).

Several workers have described a limiting effect of soil moisture on radial growth in the latter part of the season. Cantlon (1953) demonstrated that oaks on a south facing slope had a more marked "response" to rainless periods and had less radial growth in the latter part of the season than the oaks on the north facing slope. Reimer (1952) reports similar results with sugar maple. He states that the growth curves for sugar maples at the edge diverge from those for the trees in the center of the woodlot in the latter part of the season. The soil moisture regimens for these trees also show a similar divergence. He concluded that soil moisture was not critical at any time within the woodlot but dropped to a critical level during the last few weeks of the enlargement period for the trees near the south and west edges.

Friesner (1943) describes the growth curve for beech as always appearing ". . . to show a grand-period type of behavior. Environmental conditions, especially the temperature-available moisture-transpiration complex, plays a prominent part in the time of initiation, the amplitude of the curve, and the time of its cessation . . .". He (1941) compares the growth measurements of beech by Gericke in Baltimore, Maryland to his measurements:

> "In both studies growth changes were first noted coincident with the time of full expansion of the leaves, which in both instances was the middle of May. In Baltimore tree growth continued until June 5 and showed intermittent growth, correlated with rains, until the latter part of September. In the Indianapolis trees diametral increase reached its maximum rate by the middle of July. From the last date until the end of the calender year, decreases and increases alternated in correlation with rainless and rainy periods."

He further compares his growth measurements on beech in 1940 and 1941. In discussing the growth cessation he states "Growth reached a zero point in 1940 during the week ending July 15 and in 1941 during the week ending June 16. The abrupt ending of radial changes in 1941 is correlated with drought conditions." Both he (1941) and MacDougal (1938) consider the lack of soil moisture the primary factor limiting growth in the later part of the season.

Several workers (Kramer 1949) have shown that trees in poorly drained sites have shallower root systems than those on better drained sites. The author has dug pits into the different soil types in the woods and has noted a similar relationship there. In addition the roots are also more concentrated in the A horizon of the poorly drained soils.

It would therefore be reasonable to expect the results obtained in this study. The trees near depressions which have shallow root systems would show a very marked reduction in growth in the middle of a season with high spring and low summer precipitation and soil moisture. With the disappearance of water from the depressions, the soil moisture in the A horizon where the roots are most concentrated would rapidly decrease. Since the largest part of the tree roots are confined to this layer, there would be a rapid decrease in the water available to the tree and subsequently a rapid cessation of growth.

But the trees in the best drained sites with deep and less concentrated root distributions would show a more gradual reduction in growth rate as the available water is slowly reduced. Because of their better drainage, the growth cessation in these trees would begin much earlier.

The trees in less well drained sites and/or in areas of less "competition" would have a correspondingly longer growth period than either of the extreme sites. Soil moisture would neither decrease fast enough or be close enough to the wilting percent to become limiting to growth.

Two neighboring trees to the highest site which were also measured had similar but less distinct trends as the tree previously described. Another young tree near the one on the gentle rise had a similar regimen to it. Several other trees not quite as near to the depression as the tree used in this study were also measured with varying results. Some had curves similar to the tree and others appeared more like the tree on the rise. However, none of the trees near the depression had regimens like the tree on the highest site.

This variation from tree to tree is not in disagreement with the preceeding results. As was pointed out, soil moisture is affected not only by the degree of drainage and soil type but also by the degree of "competition" which is in turn dependent upon the successional relationship. Therefore, two trees on the same soil type but one in a disturbed area and the other in a mature area would have different moisture relations and consequently different growth regimens. This is quite apparent when one tries to compare the growth of trees on different sites where soil moisture has not been measured. The lack of distinct environmental and successional differences makes it difficult to group such trees into categories for statistical treatment. For this reason the author confined his discussion mainly to the three trees nearest the moisture units.

RADIAL INCREASE AND INCREMENT GROWTH

When these data are applied to the production of wood in the tree, the question arises as to how closely radial growth can be correlated with the formation of annual rings. To check this relationship, borings were made with a Swedish increment borer during January, 1953 at the same radii on which radial increases for 1952 had been measured. The cores were mounted on a grooved board and sanded with a high speed sander until the growth rings could be clearly seen. The increment growth at each radius for 1952 was measured to .001 in., and these data are compared to the dendrometer measurements in table 6.

It is apparent from the table that increment growth is directly proportional to radial growth. However, the tree with the greatest seasonal growth had the greatest "increment radial growth" while the tree with the least had the smallest. It appears that for beech the increment of xylem formation is proportional to radial growth, at least in total amount. The differences in total radial growth appear to be due more to differences in thickness of xylem than of any other tissue of the trunk.

SUMMARY

The radial increase of three canopy beech trees was measured throughout the growing season of 1952 by means of the dial-gauge dendrometer. Soil moisture

was measured with Colman fiberglas soil moisture units in the A and B horizons in near-by sites.

One tree was near a depression in a mature swamp forest community. The second tree, which was younger, was on a gentle rise and bordered an opening in the canopy. The third was in a mature beech-maple community on a slightly better drained site than the second.

The precipitation during the year was above average from January through April and below average from June through August. The average temperatures in June and July were 6° and 3° F above average.

Fluctuations appeared in the measurements during mid-summer when drought conditions were most severe.

TABLE 6

Comparison of increment growth to radial growth of three beech trees during 1952

Location	Compass Direction of Radius	Increment Growth (in thousandths of inches)	Radial Growth	*Increment* Radial Growth
Highest Site	70°	47	60	.78
	330°	38	47	.81
	200°	30	45	.67
Average		38	51	.75
On Gentle Rise	200°	109	115	.95
	20°	73	100	.73
	330°	59	83	.71
Average		80	99	.81
Near Depression	60°	20	34	.59
	200°	18	30	.60
	310°	15	25	.60
Average		18	30	.60

The growth curves in the latter part of the season were different for the trees in the various sites. These appear to be coincident with the moisture regimes. The growth of the tree near the depression which had a shallow root system was steady while the soil contained a high percent moisture. But the growth abruptly stopped when the soil moisture began to rapidly decrease in the A horizon in early July.

Growth of the more deeply rooted tree on the beech-maple or highest site had its maximum in early June followed by a gradual cessation of growth which continued into July. The soil moisture showed a corresponding gradual reduction to wilting percentage in both A and B horizons.

The growth of the younger tree in the less mature stand continued into August, apparently less affected by the drought conditions. The soil moisture on this site remained well above wilting percentage.

Differences in total radial increase were due more to differences in xylem formation than any other tissue in the stem.

ACKNOWLEDGMENTS

The writer expresses his gratitude to Dr. John N. Wolfe and Dr. Gareth E. Gilbert, Department of Botany and Plant Pathology, The Ohio State University,

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ON THE FOSSIL *CONOSTICHUS*

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The true nature and taxonomic position of the object known as *Conostichus* have long been in doubt. The question was recently revived by Dr. Kelley Hale, of Wilmington, Ohio, who has made a large collection of these forms. At the Delaware meeting of the Ohio Academy of Science on April 22, 1955, members of the Geology Section engaged in a lively discussion of the subject and some of the divergent views are here presented with the hope that additional ideas may be brought forward.

The genus *Conostichus* was erected by Lesquereux (1880) for specimens from the lower part of the Coal Measures of Pennsylvania. He described the stipe as:

"cylindrical, continuous; frond enlarging from the base upwards in the shape of a plate or of a cup, or increasing by successive, superposed layers or concentric laminae; top cup-shaped, concave.

"These organized bodies, whose reference to plants is questionable, have in their mode of growth a relation to some marine algae of our time, the Acetabulariae, which bear, on a continuous stipe, successive umbrella-shaped fronds, the lower rendered solid by incrustation of calcareous matter."

Lesquereux divided his material into three species; *C. broadheadi*, short, . . . semiglobular . . . deeply wrinkled lengthwise on the outside; *C. prolifera* with fronds thick and disciform, disposed in successive series upon a continuous narrow stipe; and *C. ornatus* (fig. 1) characterized by the regularly lobate cup, marked by deep lines diverging star-like from the axis and passing up to the top. He remarks that his examination shows that the laminae were not agglutinated, but free.

Lesquereux further supported his hypothesis of the plant origin of *Conostichus* by comparing the stellate pattern of the base with that of *Asterophycus* and by noting the peculiar, hard, whitish siliceous material of which both forms are composed. "It is a well known fact," he says (Lesquereux, 1876), "that though the algae do not take their food from the rocks to which they adhere, they often modify the composition of these rocks by their growth. Their influence . . . is even more marked upon sand or any soft substances surrounding them." He ruled out the possibility that *Conostichus* might be considered as a sponge for while there may be a degree of analogy in some species of the *Hexactinellidae* none of the sponges has a continuous axis and even in a fossil state, their surface is always rugose.

James Hall (1852) described a very similar fossil, from shaly sandstones of the Clinton group, Oneida County, New York, under the name of "Roots of *Buthotrephis*" a marine plant. He compared the cone to the depression made by the stem of the plant as it swayed to and fro. Such depressions are seen today around marine plants growing in the mud of tidal flats.

At the Delaware meeting Dr. Kenneth Caster took the position that *Conostichus* was the sand filling of the upper end of the burrow of a marine worm. He supported this opinion with the observation that certain modern marine worms make conical depressions in the sea floor by the swaying of the anterior part of their bodies while the posterior part remains in the tubular burrow below. The siliceous nature of the fossil was deemed the sand which washed into depression, possibly even accounting for the death of the worm. The longitudinal ridges are, in his view, molds of markings made by the worm's body. The successive laminae represent successive positions of the worm as it drew itself upward in its mucus-lined burrow.

THE OHIO JOURNAL OF SCIENCE 56(1): 29, January, 1956.

Dr. Wilber Stout considers *Conostichus* to be a plant which grew in fresh or brackish water. From his intimate acquaintance with Ohio rocks and his many years of study of their problems, Dr. Stout has prepared the following statement of the occurrence of *Conostichus* and his views as to its nature.

THE FOSSIL *CONOSTICHUS*

WILBER STOUT
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Conostichus is commonly associated with the fossil plant *Asterophycus*. The common horizon for their occurrence is in the shales between the Sciotoville clay and the Quakertown coal in the Pottsville series of the Pennsylvanian system.

The general section is about as follows:

	ft.	in.
Coal, *Quakertown*	1	8
Clay, impure	2	0
Shale, gray to dark, bearing *Conostichus*	33	0
Coal, impure *Anthony*, locally bearing *Lingula*		4
Clay, plastic to flint, *Sciotoville*	4	0

Some collecting places are as follows:

(1) Old clay mine on Daum hill also Nunn hill, Section 33, Harrison Township, Scioto County

(2) Land of Dr. Keyes in Section 28, Harrison Township

(3) Also near the Niner Hill and west of this on Woods Ridge

(4) In the cut of the Baltimore and Southwestern Railroad near Gephart, Section 30 and 29. Bloom Township, Scioto County

(5) On point just north of the plant of the Buckeye Fire Brick Company, Scioto Furnace, southwest Section 21, Bloom Township

(6) This horizon in Harrison and Bloom townships generally yields some fossil *Conostichus*.

At the Daum mine the strata above the Sciotoville clay are siliceous shale and bear both *Conostichus* and *Asterophycus*.

The Olive Hill, Kentucky region also bears *Conostichus*. Such fossils are not abundant.

On the front hills west of Sciotoville some of the shale above the Sciotoville clay bears sufficient manganese oxide or carbonate to give brick made from it a light brown color. Manganese minerals are a common fresh water swamp deposit.

Southwest of Scioto Furnace, about one mile from the railroad cut near Gephart, a few inches of bony shale directly above the Sciotoville clay contain *Lingula carbonia* as determined by Miss Helen Morningstar. (Bull. 25: Pottsville Fauna of Ohio, pages 21-22.) Lingulas are found in fresh, brackish and marine waters. The underlying Anthony coal is of fresh water origin, hence the shales in the interval between the Sciotoville clay and Quakertown coal may not be strictly marine, but of brackish water origin.

To the writer the *Conostichus* of the Sciotoville and Scioto Furnace areas were the most abundant and were the largest and most definitely marked of any observed throughout the entire coal fields in Ohio.

FIGURES 1 and 2. O. S. U. M. No. 10872a and 10872b, from the Pottsville series near the No. 2 Coal horizon, Sciotoville, Ohio. X .75.

Although occurring in shale the *Conostichus* fossils are composed of a fine grained sandstone. Also the same is generally true of the fossil wood—some showing cell structure found elsewhere in the coal formations, particularly in the Conemaugh series. Apparently as the vegetable matter decayed it was replaced by gelatinous silica which then crystallized.

In collecting *Conostichus* for Dr. David White of the United States Geological Survey I found a few specimens in place in the shale in the Gephart cut and at the clay mines at the Daum and Nunn hills locations. In all cases the star in the tip of the cone was down. Also there was no evidence of roots or other markings from the lower part of the cone but there were scaly layers of sandstone from the upper part. Two stars on same body were not uncommon. (See fig. 2). See Dr. David White's collection and the collection in the Geological Museum, The Ohio State University.

My conclusion is that *Conostichus* was a plant of some type growing or floating in fresh or brackish water sea.

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The Caves Beyond. *Joe Lawrence, Jr.* and *Roger W. Brucker.* Funk and Wagnalls Company, New York. 1955. xii+283 pp. $4.75.

This is the report of the Floyd Collins' Crystal Cave expedition undertaken by the National Speleological Society in 1954. The expedition consisted of 64 people from all walks of life. Some of these speleologists had explored parts of the undeveloped region of this cave before and through this expedition realized a long standing ambition.

The book is a narrative report supplemented by scientific reports in geology, biology, and hydrology. There is also a complete report on the organization and planning of the expedition so that future exploration on a large scale will be facilitated. There is a complete report on the interesting history of this cave and a short summary of Floyd Collins death in a nearby cave.

In a great part of the chapters certain phone conversations of interest are recorded verbatim so that the reader gets a feeling of being right in the deep expanse of the cave with the cavers. Although the book gives a very exciting account of the procedure and techniques involved in cave exploring, the scientific reports lack a great deal. First of all, the biology report is very incomplete and extremely general. The collecting must have been very poor and poorly recorded.

The book is well illustrated by numerous photographs taken by expedition members.

ROBERT E. WOODRUFF.

Ohio Birds and Where to Find Them. *Arthur R. Harper.* Published by Ohio Division of Wildlife, 1500 Dublin Rd., Columbus 12, Ohio. 1955. 36 pp., illus. $0.10.

This little bulletin contains a well written summary of the factors affecting Ohio birds (climate, geology, and man-made factors), a brief discussion of how to identify birds and where to look for them, and a table giving for each of 266 Ohio birds the type of habitat in which the bird is usually found and its residential status and abundance in the six major zones of the state. The table gives only common names, and does not include extinct and very rare or accidental species. The subspecies—where only one occurs, or where one predominates—is indicated in the common name (e.g., Ungava Canada Goose). The bulletin is illustrated with 2 maps, 9 photographs, and 25 line drawings.

The indication of subspecies in the common names in the table adds very little to the list's value, and since nothing is said about subspecies in the text this feature is apt to be confusing to the beginner. Ornithologists may disagree with a few of the author's statements of a bird's status or habitat preference, but these points are in general very well handled. This bulletin will be a valuable addition to the library of the Ohio bird student.

DONALD J. BORROR.

CARPIODES CYPRINUS HINEI, A NEW SUBSPECIES OF CARPSUCKER FROM THE OHIO AND UPPER MISSISSIPPI RIVER SYSTEMS.

MILTON B. TRAUTMAN[1]

Natural Resources Institute, The Ohio State University, Columbus 10

In 1817 Le Sueur (1817, p. 91–92) described *Catostomus cyprinus*, the carpsucker, from specimens obtained from the Elk River and other Cheasapeake Bay tributaries. Three years later Rafinesque (McCall's reprint, 1899, p. 113–14) in his "*Ichthyologia ohiensis*" erected the subgenus *Carpiodes* in which he placed his two newly described species of carpsuckers, namely, *Catostomus velifer* and *Catostomus carpio*, the *Catostomus cyprinus* of Le Sueur, and *Catostomus setosus* which he erroneously credited to Le Sueur as the describer. In 1855 Agassiz (1855, p. 76) gave *Carpiodes* generic rank and described a carpsucker from Lake Champlain, naming it *Carpiodes thompsoni*.

In 1896 Jordan and Everman (1896, p. 166–67) recognized *Carpiodes cyprinus*, restricting it to the Cheasapeake Bay tributaries and the Potomac River, *C. velifer* to the Mississippi valley and southwestward to the Rio Grande, and *C. thompsoni* to the Great Lakes and tributary waters. These three species and their respective ranges were generally accepted by ichthyologists until 1930, when in a revision of some catostomid fishes Hubbs (1930, p. 14–16) placed *thompsoni* in the synonomy of *C. cyprinus*, demonstrated that the name *velifer* belonged to the species long known as *Carpiodes difformis* Cope (1870, p. 480), and relegated *difformis* to the synonomy of *velifer*. Hubbs believed that the populations of *cyprinus* inhabiting the Atlantic coastal streams, Great Lakes waters and the Mississippi River system were identical. In the same publication Hubbs gave the new species name of *Carpiodes forbesi* to the description and figure of *Carpiodes thompsoni* in Forbes and Richardson's "Fishes of Illinois" (1909 and 1920, p. 79, fig. 20) and the type locality as the Illinois River drainage of Illinois.

In 1930 Osburn, Wickliff and Trautman (1930, p. 169–71) recognized only *Carpiodes cyprinus* in their revised list of Ohio fishes but in the following year Trautman (1931, p. 1) reaccepted *C. thompsoni* of the Great Lakes—St. Lawrence system as distinct from *C. cyprinus* of the Atlantic coastal and Ohio River valley drainages. In 1934 Wickliff and Trautman (1934, p. 1) reduced *C. thompsoni* to subspecific status.

In 1947 Hubbs and Lagler (1947, p. 46 and 50) first recognized two forms of *C. cyprinus*, indicating that *C. c. cyprinus* ranged from the Roanoke system of the Atlantic seaboard, northward to Lake Champlain and the St. Lawrence—Great Lakes (Lake Superior excepted) system, thence westward to southern Alberta. Realization that the Cheasapeake Bay tributaries and Lake Champlain populations were similar necessitated the placing of the name *thompsoni* in the synonomy of *C. c. cyprinus*. Unfortunately that action apparently left the population of the Ohio and Mississippi valleys without a recognizable name, despite the fact that several names for some "species" of *Carpiodes* exist. Reasons why these names are not applicable are as follows:

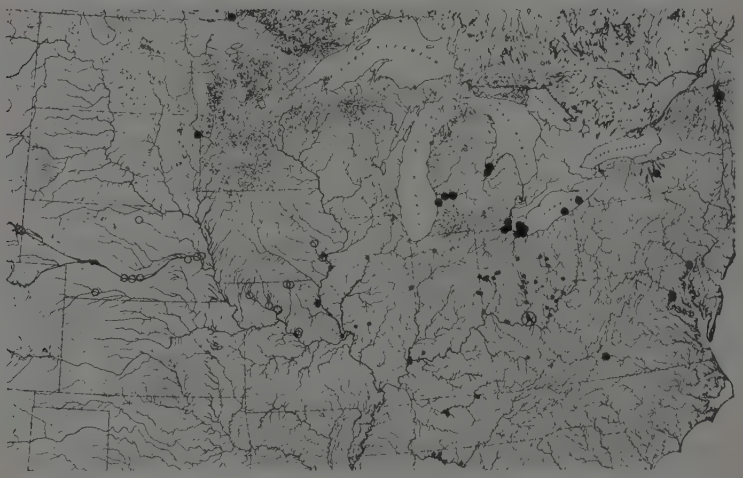
The description of *Cotostomus* (sic) *anisopterus* Rafinesque (McCall's reprint, 1899, p. 111) is unidentifiable, Hubbs (1930, p. 16) suggesting that it may refer to *Carpiodes velifer*. The great length of the dorsal fin suggests that possibility. Rafinesque described it "from a drawing of Mr. Audubon."

[1]Present Address: The Ohio State Museum, Columbus 10. Formerly with the Franz Theodore Stone Institute of Hydrobiology, where this manuscript was written.

Cope's (1870, p. 481–82) description of *Carpiodes selene* might be construed as being referable to the *cyprinus* complex, but Cope questions his type locality of "Root River, Michigan" because "the label which accompanied them [3 specimens] has disappeared." If the type locality were Michigan it would be in the Great Lakes drainage and therefore in the range of *C. c. cyprinus*, or intergrades between it and the Mississippi River system population.

Carpiodes cutisanserinus Cope (1870, p. 481) is clearly a synonym of *C. velifer*. Dr. Henry W. Fowler kindly sent me an enlarged photograph of the type which is deposited in the Academy of Natural Sciences of Philadelphia.

C. bison Agassiz (1854, p. 356), *C. damalis* Girard (1856, p. 170), *C. grayi* and *C. nummifer* (Cope, 1870, p. 482 and 484 respectively) appear to be synonyms of *C. carpio* as has long been supposed and as Hubbs (1930, p. 13–16) has indicated.



MAP 1. The type locality of *Carpiodes cyprinus hinei* is represented by a very large hollow circle. Localities for the remaining *C. c. hinei* are represented by small solid circles. Localities for *C. c. cyprinus* are represented by larger solid cirlces. Localities for *C. forbesi* are represented by medium-sized hollow circles.

Since none of the above names may be applied with any degree of certainty to the interior population of *C. cyprinus* I propose the name *Carpiodes cyprinus hinei*.

Before proceeding I wish to thank Drs. Leonard P. Schultz and Ernest A. Lachner of the U. S. National Museum (USNM), Drs. Reeve M. Bailey and Robert R. Miller of the Museum of Zoology of the University of Michigan (UMMZ) and Dr. Edward S. Thomas of the Ohio State Museum (OSM) for the privilege of examining carpsuckers preserved in their museums; Dr. Henry W. Fowler for having a photograph taken of the type of *C. cutisanserinus;* Dr. T. H. Langlois for advice; Dr. Mary A. Trautman for assistance in the taking and tabulating of all measurements, typing, etc; and particularly to Dr. Carl L. Hubbs, who since 1925 has given much encouragement to my studies of catostomid fishes and who has "gently shoved" me into describing this form.

Carpiodes cyprinus hinei, new subspecies
Central Quillback Carpsucker
(Pl., Central figure)

Types. The holotype, OSU 1889, a female, 272 mm. standard length, was collected by Theodore Maag and Russel E. Ruh, August 14, 1940, in the Scioto River, northeastern Rush Township, Scioto County, Ohio. Its body depth is

CENTRAL FIGURE: *Carpiodes cyprinus hinei* side view of the holotype.
FIGURE A: Ventral surface of head showing the absence of a knob on the symphysis of the lower jaw.

contained 2.9, its head length 3.6, and its caudal peduncle depth 7.6 times in the standard length. Its eye is contained 4.9 times in the head length and 1.6 times in the snout length. There are 7.5 scales above the lateral line and 37 scales in the lateral line.

Following are all of the paratypes, together with data:

USNM collection. 41036: 1 sp., 103 mm.,[1] Big Kanawha R., (West Virginia), 1888.

OSM collections. F81: 2 sp., 65 and 66 mm., Scioto R., Scioto Co., O., Sept. 25, 1927. F120: 2 sp., 85 and 172 mm., Scioto R., Scioto Co., O., July 14, 1928. F178: 4 sp., 65 to 75 mm., Muddy Fk., Wayne Co., O., Sept. 22, 1928. F567: 7 sp., 93 to 122 mm., Stillwater Cr., Darke Co., O., July 19, 1930. F568: 19 sp., 41 to 52 mm., Mississinawa Cr., Darke Co., O., July 19, 1930. F580: 2 sp., 90 and 108 mm., Miami R., Logan Co., O., July 26, 1930. F594: 4 sp., 51 to 57 mm., Leading Cr., Meigs Co., O., Aug. 3, 1930. F621: 6 sp., 70 to 162 mm., Scioto R., Franklin Co., O., Nov. 8, 1930. F651: 1 sp., 288 mm., Scioto R., Pike Co., O., May 18, 1931. F656: 1 sp., 171 mm., Scioto R., Pike Co., O., May 3, 1930. F659: 1 sp., 230 mm., Scioto R., Pike Co., O., May 24, 1930. F664: 1 sp., 388 mm., Buckeye Lake, Fairfield Co., O., July 1, 1930. F666: 5 sp., 213 to 253 mm., Stillwater Cr., Darke Co., O., July 19, 1930. F669: 3 sp., 331 to 335 mm., Scioto R., Pike Co., O., Sept. 14, 1930. F671: 1 sp., 257 mm., Scioto R., Franklin Co., O., Nov. 7, 1930. F676: 1 sp., 205 mm., Ohio R., Brown Co., O., Apr. 21, 1931.

UMMZ collections. 65070: 1 sp., 44 mm., Big Cr., Posey Co., Ind., Aug.–1924. 73242: 2 sp., 41 and 48 mm., Quicksand Cr., Breathitt Co., Ky., July 13, 1925. 81415: 1 sp., 72 mm., Great Pigeon Cr., near Elberfeld coal mine (Warrick Co?), Ind., Aug. 20, 1927. 87418: 1 sp., 131 mm., Little Scioto R., Crawford Co., O., May 5, 1929. 87624: 6 sp., 49 to 64 mm., Federal Cr., Athens Co., O., Aug. 17, 1929. 87639: 1 sp., 56 mm., Seneca Fk., Noble Co., O., Aug. 18, 1929. 87648: 1 sp., 205 mm., Scioto R., Pickaway Co., O., Aug. 10, 1929. 87652: 1 sp., 38 mm., Killbuck Cr., Holmes Co., O., Aug. 24, 1929. 87681: 1 sp., 67 mm., Opossum Cr., Monroe Co., O., Sept. 1, 1929. 87732: 1 sp., 208 mm., Scioto R., Pike Co., O., Sept. 22, 1929. 87807: 1 sp., 56 mm., Paint Cr., Ross Co., O., Oct. 5, 1929. 102986: 7 sp., 222 to 402 mm., Indian Lake, Logan Co., O., Aug. 15, 1932. 104300: 2 sp., 152 and 248 mm., Ohio R., Brown Co., O., Mar. 27, 1937. 104510: 6 sp., 48 to 60 mm., Mill Cr., Davidson Co., Tenn., June 20, 1936. 105687: 2 sp., 201 and 231 mm., Cumberland R., Davidson Co., Tenn., Apr.—1939. 105728: 1 sp., 102 mm., Hoops Cr., Macoupin Co., Ill., Aug. 29, 1937. 105890: 1 sp., 37 mm., Dismal Cr., Fayette Co., Ill., Oct. 4, 1937. 106467: 1 sp., 81 mm., Whitewater R., Wayne Co., Ind., Aug. 21, 1934. 106468: 1 sp., 100 mm., Noland Fk., Wayne Co., Ind., June 21, 1934. 107275: 6 sp., 198 to 247 mm., Stillwater Cr., Darke Co., O., June 19, 1930. 107276: 3 sp., 251 to 258 mm., Scioto R., Pike Co., O., Sept. 14, 1930. 107277: 3 sp., 191 to 322 mm., Scioto R., Pike Co., O., May 24, 1930. 107279: 2 sp., 275 and 295 mm., Scioto R., Franklin Co., O., Nov. 17, 1930. 107280: 1 sp., 237 mm., Scioto R., Pike Co., O., May 4, 1930. 107282: 1 sp., 315 mm., Buckeye Lake, Fairfield Co., O., Apr. 6, 1929. 107283: 1 sp., 415 mm., Buckeye Lake, Fairfield Co., O., Aug. 4, 1930. 109357: 4 sp., 37 to 50 mm., Swamp Cr., Darke Co., O., July 19, 1930. 111520: 6 sp., 61 to 107 mm., Undercliff Beach, 5 mi. N. of Henry, Putman Co., Ill., May 10, 1926. 122429: 2 sp., 138 and 146 mm., Elk R., Limestone Co., Ala., July 14, 1938. 122566: 1 sp., 138 mm., Second Cr., Lauderdale Co., Ala., July 29, 1938. 122574: 2 sp., 45 and 48 mm., Cumberland R., Clay Co., Tenn., June 22, 1939. 122590: 1 sp., 167 mm., Flint Cr., Wheeler Res., Madison Co., Ala., Sept. 10, 1938. 122596: 1 sp., 43 mm., Kettle Cr., Clay Co., Tenn., July 21, 1939. 122718: 3 sp., 141 to 166 mm., Stockton Br., Wheeler Res., Ala., Oct. 4, 1938. 122781: 1 sp., 160 mm., Sugar Cr., Wheeler Res., Limestone Co., Ala., Oct. 12, 1938. 124143: 1 sp., 39 mm., Mill Cr., Coshocton Co., O., Aug. 10, 1938. 127626: 1 sp., 325 mm., Crystal Lake, Henderson Co., Ill., July 3, 1934. 139888: 1 sp., 263 mm., Foots Pond, Gibson Co., Ind., Aug. 14, 1940. 160885: 2 sp., 58 and 66 mm., Jordan Cr., Vermilion Co., Ill., Aug. 24, 1950. 167891: 1 sp., 59 mm., Yellow R., Starke Co., Ind., Nov. 18, 1936.

OSU collections. 1085: 1 sp., 267 mm., Scioto R., Scioto Co., O., Dec. 6, 1939. 1100: 1 sp., 240 mm., Hocking R., Athens Co., O., June 29, 1939. 1133: 1 sp., 245 mm., Hocking R.,

[1]Standard length: This measurement is more desirable for preserved specimens than is fork or tail length because of the frequency of broken tails in preserved specimens.

Athens Co., O., June 27, 1939. 1135: 1 sp., 285 mm., Buckeye Lake, Licking Co., O., June 16, 1939. 1591: 1 sp., 188 mm., Scioto R., Scioto Co., O., Nov. 6, 1939. 1815: 1 sp., 253 mm., Muskingum R., Washington Co., O., Apr. 4, 1940. 1825: 1 sp., 128 mm., Muskingum R., Washington Co., O., Apr. 16, 1940. 1838: 1 sp., 215 mm., Muskingum R., Washington Co., O., Apr. 5, 1940. 1846: 1 sp., 262 mm., Muskingum R., Washington Co., O., May 9, 1940. 1850: 1 sp., 210 mm., Scioto R., Scioto Co., O., Nov. 6, 1939. 1876: 2 sp., 232 and 255 mm., Scioto R., Scioto Co., O., June 6, 1940. 6925: 7 sp., 38 to 53 mm., Whitewater R., Hamilton Co., O., Aug. 14, 1946. 7607: 10 sp., 22 to 27 mm., Yellowbud Cr., Pickaway Co., O., July 3, 1948. 9135: 1 sp., 277 mm., Big Walnut Cr., Franklin Co., O., May 10, 1950.

Diagnosis. As indicated in Table 1, when measured into standard length *C. c. hinei* differs on the average from *C. c. cyprinus* in having a less deep and more rounded body, a slightly longer head, and a less deep caudal peduncle. Measured into head length the eye of *C. c. hinei* is larger, and when the eye is measured into snout length the snout is shorter in *C. c. hinei* than in *C. c. cyprinus*. The number of scale rows extending obliquely backward and upward from the lateral line to the dorsal fin origin averages 7.4[2] in *C. c. hinei* and 8.0 in *C. c. cyprinus;* the number of scales in the lateral line averages 36.8 in *C. c. hinei* and 37.5 in *C. c. cyprinus*.

No significant differences between *C. c. hinei* and *C. c. cyprinus* were noted in the length of the upper jaw when measured into head length, the number of rays in the dorsal fin, or in the length of the anterior rays of the dorsal fin.

As a species *C. cyprinus* differs from *C. forbesi* in having the anterior rays of the dorsal fin in large young and adults extending nearly to or beyond the posterior end of the dorsal fin base, whereas in *C. forbesi* the anterior rays extend little if any beyond the middle of the fin. When measured into the standard length *C. cyprinus* differs from *C. forbesi* in having a deeper body, a shorter head and a less deep caudal peduncle. Measured into head length the eye of *C. forbesi* averages slightly longer than in *C. c. hinei* and definitely longer than in *C. c. cyprinus*, and when the eye of *C. forbesi* is measured into snout length, the snout length averages the same as in *C. c. hinei* but is shorter than in *C. c. cyprinus*.

C. forbesi may have a slightly shorter upper jaw than has *C. cyprinus* when measured into head length. The number of scales above the lateral line in *C. forbesi* averages 7.0; it is 7.4 in *C. c. hinei* and 8.0 in *C. c. cyprinus*. The number of scales in the lateral line in *C. forbesi* averages 36.6 which is comparable to 36.8 in *C. c. hinei* and fewer than 37.5 in *C. c. cyprinus*.

C. forbesi and *C. cyprinus* differ from *C. carpio* and *C. velifer* in lacking the knob, present in large young and adults, on the symphysis of the lower jaw.

Variations in body proportions. Species in the genus *Carpiodes* have few specific and subspecific characters and under different environmental conditions these few are subject to extreme variations. Furthermore, these characters are undeveloped in some young less than 40 mm. in standard length making identification of some of these as to species impossible.

Variations in body proportions and length-weight relationships within a species are especially great and often are very marked in specimens between one body of water and another and from one season or year to another. These variations have caused great diversity of opinion among ichthyologists as to the number of species within the genus, and which of the available "species" descriptions should or could be recognized.

When growth is unusually rapid and/or conditions appear to be optimum, some specimens of *C. c. hinei* approximate *C. c. cyprinus* in body proportions, and especially in autumn when their coefficiency of condition is greatest. However, these extremely fat, robust individuals of *C. c. hinei* differ from *C. c. cyprinus* in having thicker, broader bodies which are broadest above or along the lateral lines

[2]When the scale nearest the dorsal fin or ridge is smaller than the others its depth is compared with the normal-sized scale immediately below and is measured to the nearest tenth.

TABLE 1

Measurement of the two subspecies of Carpiodes cyprinus and of Carpiodes forbesi. In the body of the table figures without parentheses for each rubic are extremes, those immediately following in parentheses are averages

Length class (mm.)	20–35	36–100	101–200	201–300	301–420	20–420 *
Specimens*						
cyprinus	10	35	15	28	15	103
hinei	10	94	37	44	9	194
forbesi	8	27	11	1	1	48
Standard length (mm.):						
cyprinus	22–25 (23)	45–95 (75)	122–200 (168)	203–295 (257)	303–420 (338)	22–420 (171)
hinei	22–27 (24)	37–100 (57)	101–198 (140)	202–295 (245)	311–415 (349)	22–415 (127)
forbesi	26–35 (32)	37–97 (54)	114–195 (135)	258	332	26–332 (86)
Body depth in standard length. †						
cyprinus	2.9–3.4 (3.1)	2.3–2.9 (2.7)	2.2–2.7 (2.5)	2.3–2.8 (2.6)	2.3–2.8 (2.5)	2.2–3.4 (2.6)
hinei	3.1–3.7 (3.4)	2.5–3.9 (3.0)	2.5–3.2 (2.8)	2.5–3.2 (2.8)	2.7–3.1 (2.9)	2.7–3.9 (2.9)
forbesi	3.2–3.7 (3.5)	2.9–3.5 (3.3)	2.9–3.3 (3.1)	3.4	3.2	2.9–3.7 (3.2)
Head length in standard length:						
cyprinus	2.6–2.8 (2.7)	2.9–3.6 (3.3)	3.3–3.7 (3.5)	3.4–3.9 (3.7)	3.6–3.8 (3.7)	2.6–3.9 (3.4)
hinei	2.5–2.7 (2.6)	2.7–3.8 (3.0)	3.1–3.7 (3.4)	3.4–4.0 (3.6)	3.6–4.3 (3.9)	2.5–4.3 (3.2)
forbesi	2.5–2.8 (2.7)	2.6–3.3 (3.0)	3.1–3.5 (3.3)	3.8	3.7	2.5–3.8 (3.0)
Eye length in head length:						
cyprinus	4.1–4.6 (4.4)	4.0–5.6 (4.5)	4.8–6.1 (5.4)	5.0–7.0 (5.9)	4.9–8.8 (6.2)	4.0–7.0 (5.3)
hinei	3.0–3.6 (3.4)	3.1–4.9 (3.9)	3.8–4.9 (4.4)	4.4–5.6 (4.9)	4.8–5.8 (5.3)	3.0–5.8 (4.3)
forbesi	3.3–4.2 (3.8)	3.7–4.8 (4.2)	4.1–5.6 (4.6)	4.9	4.9	3.3–4.9 (4.2)
Eye length in snout length:						
cyprinus	1.2–1.4 (1.3)	1.1–1.7 (1.3)	1.5–1.9 (1.7)	1.6–2.3 (1.9)	1.7–2.7 (2.1)	1.1–2.7 (1.6)
hinei	1.0–1.2 (1.1)	0.8–1.3 (1.1)	1.1–1.7 (1.3)	1.2–1.8 (1.5)	1.6–1.8 (1.7)	0.8–1.8 (1.2)
forbesi	1.0–1.2 (1.1)	1.0–1.3 (1.2)	1.1–1.7 (1.4)	1.6	1.8	1.0–1.8 (1.2)
Upper jaw length in head length: ‡						
cyrinus	3.6–4.4 (3.8)	3.9–5.4 (4.6)	4.4–6.1 (5.1)	4.0–6.2 (5.1)	4.5–5.7 (5.1)	3.6–6.2 (4.8)
hinei	3.7–3.9 (3.8)	3.7–5.7 (4.6)	4.3–5.8 (5.1)	4.1–5.4 (4.9)	4.3–5.5 (5.1)	3.7–5.8 (4.7)
forbesi	4.0–5.3 (3.4)	4.2–5.6 (5.0)	4.8–7.3 (5.8)	4.9	4.5	4.0 7 3 (5.0)
Caudal peduncle depth in standard length:						
cyprinus	5.9–6.9 (6.3)	5.9–7.9 (6.8)	6.1–7.6 (6.9)	6.6–7.9 (7.1)	6.7–7.9 (7.2)	5.9–7.9 (6.9)
hinei	6.7–7.4 (6.8)	6.4–8.4 (7.3)	6.5–7.9 (7.3)	7.0–8.4 (7.7)	7.2–8.1 (7.9)	6.4–8.4 (7.4)
forbesi	6.7–8.3 (7.6)	7.1–8.5 (7.7)	7.7–8.6 (8.0)	8.3	7.5	6.7–8.6 (7.7)

*Measurements of *C. c. cyprinus* and *C. forbesi* are from specimens deposited in the OSM, UMMZ, USNM and OSU collections. Measurements of *C. c. hinei* are from the paratypes mentioned above together with measurements of specimens, not paratypes, collected west of the Mississippi River; these latter were tabulated separately but when no significant differences between these and specimens taken east of the Mississippi were found they were included in the table.

†All measurements and counts were taken to the nearest tenth of a mm. The division of the measurement of one body part into a larger body part was done with the aid of a calculating machine and therefore is an arithmetic ratio, not a "step" measurement. (Step measurement is taken with dividers by measuring a body part, then "stepping off" with dividers this part into a larger part, such as head length into standard length.) In this group of fishes the arithmetic ratio can be used as a step measurement for identification of the average specimen of moderate or large size.

‡Upper jaw length was measured carefully by inserting one point of the dividers through the skin to the posterior-most point of the maxillary and then firmly pressing the other point against the tip of the upper lip (with mouth closed) so that it pressed against the anterior-most point of the premaxillary.

and their predorsal ridges are broadly rounded, whereas individuals of *C. c. cyprinus* have more slab-sided bodies with the broadest portions slightly below the lateral lines and the predorsal ridges are rather sharply angular.

On the other hand, when growth is retarded and/or conditions are submarginal, as when individuals are trapped in pools of non-flowing waters, some specimens of *C. c. hinei* approximate *C. forbesi* in body proportions. These individuals have very slender bodies and large eyes. It was from such specimens that Dr. Carl L. Hubbs and I concluded in 1934 that *C. forbesi* was present in some streams in Ohio and so they were recorded (Wickliff and Trautman, 1934, p. 1). Later we realized our mistake and *C. forbesi* was removed from the list of Ohio fishes.

Ranges and Intergradations. As partly shown on Map 1, *C. c. hinei* inhabits in typical form all of the Ohio River system, and the Mississippi River system in Illinois and the eastern parts of Missouri and Iowa.

C. c. cyprinus ranges from the Roanoke River system northward along the Atlantic coastal drainages to Lake Champlain and the St Lawrence River system, thence westward through the four southermost Great Lakes. Intergradation between *C. c. cyprinus* and *C. c. hinei* occurs in the southern tributary streams and embayments of Lake Erie. No well-marked intergradation was noted in the Ohio River drainage of northern Ohio. Examination of the scanty material from west of the Great Lakes indicates that specimens from the Lake of the Woods in Minnesota and from northeastern South Dakota appear to be referable to *C. c. cyprinus* and that specimens from the upper Mississippi River drainage of northern Iowa, southern Minnesota and southern Wisconsin seem to be intergrades. More specimens are needed to determine the subspecific status of individuals from this region. I have seen no specimens of *C. cyprinus* from southern Missouri southwestward.

C. forbesi appears at present to be largely restricted to the lower Missouri River drainage, including the Platte, and to Mississippi tributary streams in Iowa.

It is with deepest appreciation that I name this form in memory of the late Professor James Stewart Hine (Kennedy, 1931, p. 510–11). From 1922 until his death on December 22, 1930, and particularly between 1925 and 1930 while he was Curator of Natural History of The Ohio State Museum, he unstintingly gave of his time and energy to teach me the fundamentals of biology and aided in many ways in my investigations of Ohio waters.

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Introduction to Physical Geology. *C. R. Longwell* and *R. F. Flint.* John Wiley and Sons, Inc., New York. 1955. x+432 pp. $4.95.

On the dust jacket this book is advertised as completely new, although in the tradition and spirit of the three earlier texts in which these authors participated. For them to make a complete break from the earlier series would be difficult if not impossible, as any user of textbooks knows. Nevertheless, the publisher's claim is in the main true. The present text is only two-thirds as long as the old 3rd edition, which will be a great boon to both the professor and student, especially in courses of quarter length. The order of presentation of subjects has been revised to a form much like that in most of the current leading geology textbooks: i.e., introductory material followed by methods and scope of the science, materials of the crust, geologic time, volcanic and igneous geology, weathering, gradational processes, sedimentation, structure, metamorphism, mountain-building, and mineral resources. This new arrangement probably makes it possible for most teachers to assign the chapters in numerical order and to take advantage of the authors' own transitions from chapter to chapter. All but one of the chapters from the earlier work have been shortened. Although many retain the same plan of discussion and exemplification the language has been thoroughly worked over to make it more direct, simple, and intelligible. The effusive exposition that was a trademark of the earlier works is absent from this text.

Much of the burdensome descriptive material of the old book has been put in diagram form. The authors have retreated from their former practice of pigeonholing all sorts of landforms into youthful, mature, and old: this classification is retained only for surfaces developed by streams. Whereas the older work was deficient in illustrations and had almost no diagrams, the new one is abundantly provided with both. Particularly helpful to the uninitiated is the practice of marking labels directly on the photographic illustrations. Unfortunately, a number of the photographs are poorly reproduced. All classification of minerals and rocks is placed in appendices.

In addition to the general improvement of exposition throughout the book, specific gains have been made in some chapters. Chapter 3 is a new discussion of minerals and rocks, and also includes an introduction to mapping and structure sections. Chapter 4, on geologic time, is new. Chapter 6, on weathering and soils, now includes some basic meteorology. Chapter 10, on ground water, now has a quantitative approach, and although it describes and illustrates permeability, no mention is made of porosity and the distinction between the two. The treatment of lakes and basins (Chapter 11) has been improved by condensation and by putting it on a "case-study" basis. The section on arid lands and their typical processes (Chapter 13) has been expanded and combined into one chapter with work of the wind. Physical oceanography and submarine geology have been improved and expanded and put in a separate chapter (14) from the material on waves, currents and shore processes (15). Unfortunately, the latter no longer presents a classification of coasts and entirely ignores coral reefs. Probably the outstanding single improvement in the new text is the chapter on metamorphism (18).

Unlike the forerunners of this text, emphasis is placed upon processes and examples, with a more quantitative approach in some parts. Detailed examples and classifications are left out and are thus at the discretion of the class lecturer. These qualities make the text more flexible and probably more widely useful than its predecessors.

MALCOLM P. WEISS

FACTORS AFFECTING THE DEGRADATION PROCESSES FOR DEXTRAN

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The usefulness of dextran as a plasma volume expander is highly dependent upon its molecular weight and homogeneity. Grönwall and Ingelman (1945) reported that the molecular weight of the native dextran is in the order of many millions and that its use in this form will cause injurious reactions.

Ingelman and Halling (1949) found that the high molecular weight dextran enhances the sedimentation tendency of the red blood corpuscles and that the lower the molecular weight of the polymer, the less is this action.

In the production of such a material for use as a blood expander, it is necessary either to control the fermentation to produce the desired molecular size as reported by Hehre (1953), Koepsell *et al.* (1953), *Nadel et al.* (1953), Spendlove (1953) and Tsuchiya *et al.* (1953) or degrade the high molecular weight dextran as established by Grönwall and Ingelman (1945) (1948) (1953), Ingelman (1948), Lockwood *et al.* (1951), Renfrew and Cretcher (1949), Stacey (1951), Stacey and Pautard (1952), Stoycos (1954), Tsuchiya *et al.* (1952), Whiteside-Carlson and Carlson (1952), Wolff *et al.* (1953 and 1954), to a value where the majority of the degraded polymer will have molecular weights in the order of the plasma proteins (about 75,000 ± 25,000) as reported by Pulaski (1952).

This investigation was concerned with the study of the factors affecting the production of degraded dextran and the molecular weight distributions obtained using different procedures of depolymerization, namely, acid hydrolysis, enzyme degradation and ultrasonic vibration.

MATERIALS AND METHODS

Preparation of Dextran Samples

Culture. *Leuconostoc mesenteroides* N.R.R.L. B–512 used. Dextran produced by this organism has been previously characterized by Wilham *et al.* (1953). The culture was kept under refrigeration at 6° C and transfers were made every three weeks. For dextran production the culture was activated by several passages through the same media as used for the fermentation procedure, allowing 12 hr. incubation between each inoculation.

Media. For the stock culture, the medium used had the following composition per liter: sucrose 40.0 g., yeast extract 1.0 g., acid hydrolyzed casein 5.0 g., K_2HPO4 5.0 g., NaCl 2.0 g., $MgSO_4$ 0.022 g. and agar 15 g. For the production of dextran, the previous medium was used except that the sucrose content was 150.0 g. and agar was omitted.

Sterilization, inoculation and incubation. Sterilization of media, effected by autoclaving at 15 lbs. for 15 min., was followed by cooling and inoculation with the active culture. For the production of high molecular weight dextran, the culture medium was incubated at 25° C for 3 days, after which the dextran was precipitated.

Precipitation of dextran. The fermented liquor was diluted, necessary to decrease viscosity, with distilled water and filtered through large bacterial filters (Seitz or Berkefeld). Isopropyl alcohol was added to the filtrate to give 60 percent by volume, and the mixture was kept in the refrigerator for 20 hr. The supernatant solution was then decanted and the concentrated gel phase of the dextran was added drop-wise to 99 percent isopropyl alcohol while

the alcohol was stirred vigorously in a Waring Blendor. By use of this procedure the gummy stage was avoided and the dextran was precipitated as a very fine flocculent powder, which was separated from the alcohol by filtration. Mechanical stirring of the precipitated dextran was repeated in 99 percent isopropyl alcohol after which the dextran was filtered and dried in vacuum over anhydrous calcium chloride at 25° C.

Measurement of dextran concentration. The dextran solutions for viscosity measurements were made up to known concentrations in volumetric flasks by adding a known weight of dried dextran and filling to the mark with distilled water. The concentration of each solution was checked with the Bausch and Lomb dipping refractometer.

Depolymerization Methods

Enzymatic degradation. Production of enzyme degraded dextran B–512 is based on incubation of the fermented media, extended beyond the time for maximum viscosity, as reported by Jeanes *et al.* (1948). A six liter Erlenmeyer flask, containing 5.5 lit. of sterile medium, was inoculated with an active culture of *L. mesenteroides* B–512 using 5 percent inoculum. The flask was incubated for 3 wk., during which time it was shaken at intervals of 3 days. The fermented medium was diluted and clarified by passage through a bacterial filter. The degraded dextran was recovered by precipitation using isopropyl alcohol.

Acid hydrolysis. Partial acid hydrolysis of a 6 percent solution of high molecular weight dextran was accomplished using 0.1 N HCL at 60° C in a water bath. The hydrolysis was followed by measuring the relative viscosity of the product. The degradation was stopped by cooling and neutralization with 5 N NaOH at relative viscosities between 2.0 and 3.0. The partially hydrolyzed dextran was precipitated as previously described.

Ultrasonic depolymerization. In this investigation two types of apparatus were used, a model U–300 Ultrason and a Radio Sonorator model RS–2 operating at frequencies of 450,000 and 250,000 cycles per second, respectively.

For the degradation process, the aqueous solution of dextran was subjected to the ultrasonic waves. Irradiation was made in 15 min. periods since it was necessary to stop the ultrasonic treatment to cool the quartz crystal.

Fractional Precipitation

Degraded dextran consists of various molecular weights. Fractionation experiments using isopropyl alcohol were conducted to investigate the molecular weight distribution and to obtain homogeneous dextran fractions for physicochemical studies. The optimum conditions for fractional precipitation of dextran with isopropyl alcohol were studied and a procedure was developed for the separation of the two liquid phases which are formed when alcohol is added to dextran solution, up to alcohol concentration of 50 percent by volume.

(1) An aqueous solution of low concentration of dextran (1–3 percent) was used, since, in a series of fractionation experiments, it was found that the lower the dextran concentration, the more efficient was the fractionation procedure.

(2) Under mechanical stirring, the desired amount of isopropyl alcohol was added slowly, while the temperature was kept at 10 to 12° C. This temperature was chosen to permit the use of lower alcohol concentrations in the precipitation of dextran.

(3) The temperature of the solution was raised to 25 to 30° C to redissolve the precipitated fraction and any low molecular weight dextran which might be precipitated by momentarily high local concentration of alcohol occurring during step 2. Then the clear solution was cooled to 10 to 12° C and allowed to stand overnight at this temperature.

(4) To separate the gel phase from the supernatant solution, the system was cooled quickly to 0 to 2° C to cause the gel phase to freeze and adhere to the

bottom of the bottle. "A cloudiness or a pseudo-fraction appeared in the upper liquid phase on cooling at 4° C but did not settle out. On subsequent warming of the separated supernatant solution to 10 to 12° C, the cloudiness disappeared." The upper liquid phase, which does not freeze because of its higher alcohol content, was decanted easily, insuring a clean-cut separation.

(5) Lower molecular weight fractions were obtained by repeating steps 2 through 4 on the supernatant solutions from the preceding fractionations. By measuring the volume of the supernatant solution, the amount of alcohol necessary to precipitate the next fraction was calculated.

(6) Each fraction was redissolved in warm water (50° C) and the dextran samples were then precipitated with isopropyl alcohol, dried and weighed. Each fraction was sub-fractionated in the same manner as outlined in steps 1 through 5.

Viscosity Measurements

The viscosity measurements were made at 25° ± 0.05° C using a No. 50 Ostwald-Fenske viscosimeter and dextran concentrations between 0.2 and 0.6 percent. Specific viscosities were obtained from the relationship,

$$\eta sp = \eta/\eta_o - 1$$

where

η = viscosity of dextran solution
η_o = viscosity of distilled water

Intrinsic viscosities, $[\eta]$ in deciliters/gram, were obtained by plotting the reduced viscosity, $\eta sp/c$, versus concentration, c, in percent by weight, and extrapolating the reduced viscosity values to zero concentrations.

Light-Scattering

The instrument used in this investigation was the B–S light-scattering photometer designed by Brice *et al.* (1950). The refractive index increments were determined with the B–S differential refractometer. Slight amounts of dust particles scatter light considerably and give rise to erroneous results. Consequently, the dextran solutions were filtered through an ultrafine sintered glass filter into a semi-octagonal cell and light-scattering measurements were made at 0, 45, 90 and 135 degrees to the emergent beam for both blue and green light to obtain values for both turbidity, τ, and dissymetry, Z. The weight-average molecular weight was then calculated using the following relationship which was established by Debye (1941) and Debye *et al.* (1946):

$$H\,(c/\tau) = 1/M + 2Bc$$
$$H\,(c/\tau)_{c=o} = 1/M$$

Where H is the refraction constant, M is the solute weight-average molecular weight, B is a constant depending on the solvent and "c" is the concentration in grams per milliliter. The turbidity, τ, was made using dextran concentrations between 0.03 and 0.6 percent.

RESULTS

Enzymatic Degradation

The following studies were carried out to investigate the molecular weight distribution of enzyme degraded dextran (Hamdy, 1953) previously described by Jeanes *et al.* (1948) as "autolyzed" dextran.

Two liters of 2 percent aqueous solution of that dextran with an $[\eta]$ value of 0.33 were subjected to fractional precipitation. Five main fractions were obtained (E_1–E_5) at the following alcohol concentrations: 32.5, 37.5, 42.5, 47.5 and 55.0 percent. The percentage yields of the fractions were calculated and their intrinsic viscosities were determined. This experiment was duplicated with almost the same results and the averages are recorded in table 1. The first two fractions E_1 and E_2 were further separated into six and four subfractions, respectively.

Intrinsic viscosity measurements were performed on all the fractions and subfractions while light-scattering experiments were carried out on some of each, and the results are presented in table 2 indicating a wide range of molecular weights.

Acid Hydrolysis

Studies using partial acid hydrolysis were made to investigate the effects of certain factors on the degradation and the molecular weight distribution.

Effect of acid concentration and time. A 6 percent solution of high molecular weight dextran (B–512) was hydrolyzed in different concentrations of hydrochloric acid (0.05, 0.10, 0.15 and 0.20 N) in a water bath at 60° C. The hydrolysis was followed by relative viscosity measurements of the dextran solution at different

TABLE 1

Results of fractional precipitation of two liters of 2 percent aqueous solution of enzyme degraded dextran B-512 with an [η] value of 0.33

Fractions	% IPA	wt of fractions in g.	% yield*	[η]	Mol. Wt.**
E_1	32.5	19.8	49.5	0.48	———
E_2	37.5	5.8	14.5	0.36	129,600
E_3	42.5	2.7	6.8	0.27	100,000
E_4	47.5	2.4	5.9	0.18	48,000
E_5	55.0	1.6	4.0	0.14	27,000

*Percent yield, based on the initial weight of the fractionated dextran.
**Molecular weight as determined by light-scattering.

TABLE 2

Molecular weight distribution of enzyme degraded dextran B-512

Fraction	Subfraction	% IPA	% yield	[η]	Mol. Wt.
E_1	a	30.0	37.4	0.68	———
	b	32.5	1.4	0.58	500,000*
	c	35.0	4.0	0.50	355,000‡
	d	37.5	3.4	0.35	175,000*
	e	45.0	1.3	0.25	89,000‡
	f	55.0	1.0	0.17	43,500*
E_2	a	35.0	10.8	0.39	214,000‡
	b	37.5	1.8	0.26	96,000*
	c	45.0	0.8	0.20	57,000*
	d	55.0	0.8	0.10	14,000‡

*Obtained by light-scattering method.
‡Calculated from intrinsic viscosity and the equation $M = 1.42 \times 10^6 \times [\eta]^2$ (Hamdy, 1953).

time intervals. Figure 1 presents the results of the measurements, which indicate that the rate of decrease of viscosity is greatest at the higher acid concentrations and is most rapid in the early stages of hydrolysis.

Effect of temperature. Hydrolysis of 6 percent solutions of dextran in 0.1 N hydrochloric acid was effected in a water bath at temperatures of 45°, 70°, 80° and 98° C., respectively. The hydrolysis was followed by viscosity measurements at 20 min. intervals. The results, recorded in table 3 show that the resulting rate of drop in viscosity increases with increasing temperature.

Molecular weight distribution. Two liters of 2 percent aqueous solution of partially acid-hydrolyzed dextran, which had an intrinsic viscosity of 0.19, were fractionated to determine molecular weight distribution. Intrinsic viscosity measurements were made on the fractions and the results are given in table 4. All the fractions, with the exception of G_3, were refractionated. Intrinsic viscosity

measurements were made on all the sub-fractions while light-scattering determinations were performed on some of them; the results recorded in table 5 indicate a wide range of molecular weights.

Ultrasonic Depolymerization

Procedure. A 6 percent solution of dextran in distilled water was subjected to ultrasonic vibrations for a total period of 4.0 hr., using model U–300 ultrason, a voltage input of 2.2 k.v. and a current input of 250 milliamps, *i.e.*, a power input of 550 watts. The degradation was followed by viscosity measurements as shown in table 6. The degraded polymer was recovered and its intrinsic viscosity was found to be 0.65. Preliminary fractionations were performed on 500 ml. of a 2 percent aqueous solution of the degraded polymer. The yields of the fractions

TABLE 3

The effect of temperature on the hydrolysis of dextran by 0.1 N hydrochloric acid

TIME OF HYDROLYSIS,	RELATIVE VISCOSITY OF 6% SOLUTION AT VARIOUS TEMPERATURES			
MIN.	45. C	70. C	80. C	98. C
0	23.0	23.0	23.0	23.0
20	21.4	12.2	11.0	1.7
40	21.2	5.8	5.7	1.3
60	21.0	4.3	3.7	1.3
80	20.5	3.5	3.4	1.3
100	19.8	3.1	2.6	1.3
120	19.2	2.8	2.4	1.3

TABLE 4

*The intrinsic viscosity and molecular weight distribution of various fractions of acid hydrolyzed dextran B-512**

Fraction	% IPA	wt. of fraction in g.	% yield	$[\eta]$	Mol. Wt.**
G_1	35.0	13.2	33.0	0.30	127,000
G_2	40.0	8.7	21.8	0.19	55,000
G_3	45.0	9.4	23.6	0.16	33,500
G_4	50.0	4.1	10.3	0.11	17,500
G_5	55.0	2.2	5.5	0.09	12,000

*This acid hydrolyzed dextran had an intrinsic viscosity of 0.19.
**Obtained by light-scattering method.

were calculated and their intrinsic viscosities determined. The results are given in table 7, which, with table 6 show that: (1) high molecular weight dextran is depolymerized by ultrasonic vibrations using a power input of 550 watts; (2) a total of 83.6 percent of the degraded polymer had an intrinsic viscosity value of almost the same as that before fractionation; (3) the intrinsic viscosity of the degraded dextran was greater than the desired value (0.255 ± 0.035) for a plasma volume expander. Therefore, the following experiments were conducted to investigate certain factors affecting the ultrasonic depolymerization.

Effect of dextran concentration. Various concentrations of aqueous solutions of dextran were subjected to ultrasonic vibrations using a power input of 430 watts. Depolymerization was followed by viscosity measurements; the results are given in figure 2.

Effect of the molecular weight of dextran. The previous experiment showed that the ultrasonic treatment of dextran led to a large drop in viscosity in the first 60 to 90 min., followed by a small decrease beyond 90 min. The following experiment was conducted to attempt to explain this observation.

A native dextran with a specific viscosity of 1.87 for a one percent aqueous solution and an enzyme degraded dextran with a specific viscosity of 0.45, also in one percent solution, were subjected to the same treatment at a frequency of 450 k.c., using a power input of 430 watts. Depolymerization was followed by viscosity measurements. The results (figure 3) show that the high molecular weight dextran was degraded rapidly, while the low molecular weight polymer was only slightly affected by the depolymerization process. Therefore, it can be stated that the ultrasonic treatment acts on the large molecules of dextran much more rapidly than on the small molecules.

Effect of power input. In the previous experiments, it was found that the dextran concentration and its molecular weight are important factors in the degradation process and with high concentrations and high molecular weights, a longer period of exposure to ultrasonic waves was required to reach the desired molecular size. It was decided, therefore, to study the effect of power input (intensity) of the ultrasonic vibration on the degradation of dextran.

TABLE 5

The intrinsic viscosity and molecular weight distribution of various sub-fractions of acid hydrolyzed dextran B-512

Fraction	% IPA	Subfraction	%** yield	$[\eta]$	Mol. Wt.
G_1	32.5	a	7.5	0.38	205,000*
	35.0	b	9.2	0.30	127,800
	40.0	c	6.1	0.22	69,000
	45.0	d	1.1	0.19	55,500
	55.0	e	7.5	0.16	36,000*
G_2	37.5	a	13.5	0.28	111,000
	40.0	b	1.1	0.21	63,000*
	42.5	c	2.3	0.18	46,000
	45.0	d	1.4	0.15	32,000*
	50.0	e	0.5	0.12	20,000
	55.0	f	1.4	0.08	9,000*
G_3	40.0	a	9.5	0.17	41,000
	42.5	b	6.0	0.15	32,000
	50.0	c	2.3	0.12	20,000
	55.0	d	2.6	0.06	51,000*
G_4	42.5	a	4.0	0.14	28,000*
	45.0	b	1.4	0.11	18,000*
	50.0	c	1.6	0.09	12,000
	55.0	d	1.4	0.04	2,500*

*Calculated from intrinsic viscosity and the equation $M = 1.42 \times 10^6 \times [\eta]^2$ (Hamdy, 1953).
**Yield based on the original material for fractionation.

Two portions of a 2 percent aqueous solution of high molecular weight dextran were subjected to ultrasonic vibrations. One part was irradiated using a power input of 430 watts for a period of 3.5 hr. The other part was treated for the same length of time with a power input of 630 watts. Degradation was followed by viscosity measurements. The results are shown graphically in figure 4 which indicates that: (1) the initial rates of degradation as measured by reduced viscosity are approximately the same for power input of 430 and 630 watts; (2) for long irradiation times the molecular sizes produced by 630 watts are smaller than those produced by 430 watts.

Molecular weight distribution. Approximately 50 g. of high molecular weight dextran were subjected to ultrasonic vibration using a Radio Sonorator model RS-2 under the following controlled conditions: (1) the use of 4 percent aqueous solution; (2) the use of 430 watts power input for the first period of degradation (8 hr.); and (3) the use of 600 watts power input for the second period of degradation (8.5 hr.); to reach an intrinsic viscosity value of 0.24–0.26.

The degraded polymer was subjected to fractional precipitation using two liters of 2 percent aqueous solution.

Five main fractions were obtained using varying concentrations of isopropanol. Table 8 presents the data for the percentage yield and the intrinsic viscosities of the fractions, indicating that 94.0 per cent of the degraded dextran was in the range of that suitable for a plasma expander, and that 92.0 percent of the frac-

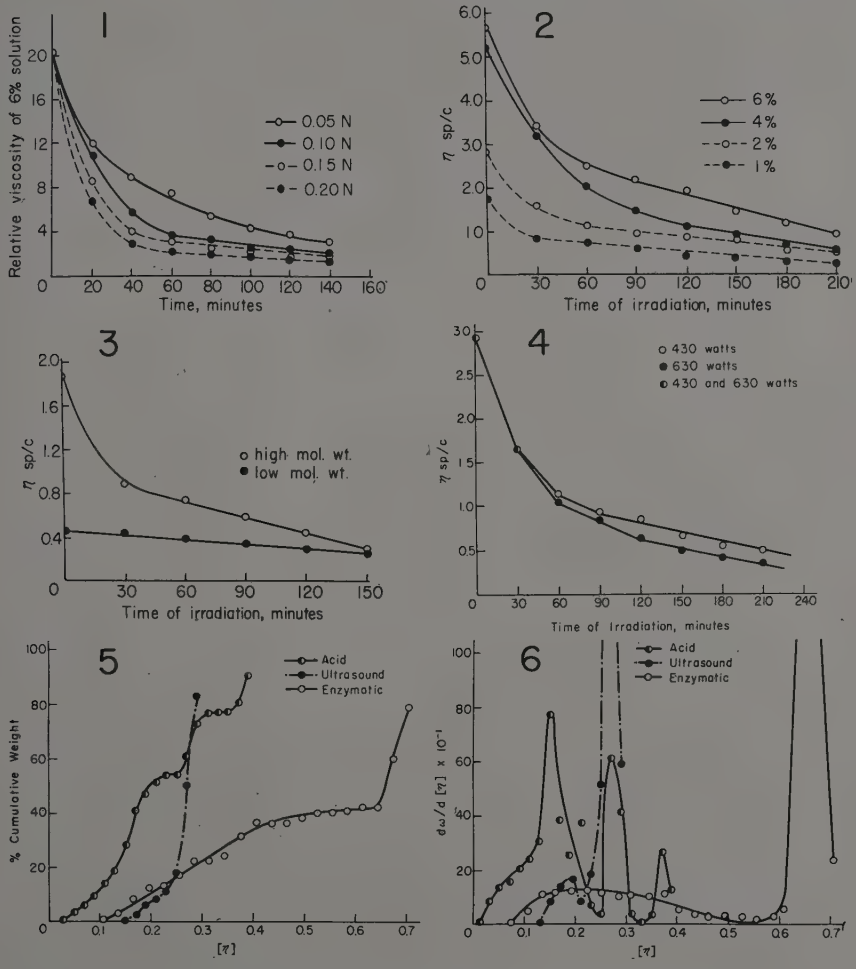

FIGURE 1. Effect of acid concentration on the hydrolysis of dextran.
FIGURE 2. Effect of dextran concentration on the ultrasonic depolymerization.
FIGURE 3. Effect of molecular weight of dextran on the ultrasonic degradation using 1% aqueous solution of dextran.
FIGURE 4. Effect of energy input on the degradation of dextran B-512 by ultrasound.
FIGURE 5. Comparison of integral distribution curves for degraded dextran B-512 resulting from different methods of depolymerization.
FIGURE 6. Comparison of differential distribution curves of degraded dextran B-512 resulting from different methods of depolymerization.

tionated polymer had almost the same [η] as that of the unfractionated polymer. Further fractionation was carried out on fractions U_1 and U_2 and the data for percentage yields of the sub-fractions (based on the original weight of the degraded dextran) and their intrinsic viscosities are recorded in table 9. It may be seen that most of the dextran is precipitated in the first and second sub-fractions, and that the intrinsic viscosities for the majority of the sub-fractions are in the range desired for a plasma volume expander.

Reducing power of degraded dextran. The reducing abilities of the polymers, resulting from the three different procedures of degradation, were investigated using Benedict solution. It was found that the acid and enzyme degraded polymers had markedly high reducing powders in contrast to the ultrasonically depolymerized dextran which was non-reducing.

TABLE 6

The effect of ultrasonic vibrations in reducing the viscosity of an aqueous solution of dextran B-512 using a power input of 550 watts

Time of irradiation, min.	ηsp/c*
0	5.88
30	3.30
60	2.50
90	2.30
120	2.00
150	1.20
180	0.89
210	0.69
240	0.67

*η sp/c values are for dextran concentration of 6%, where η sp = η rel.-1.

TABLE 7

Intrinsic viscosity distribution of an ultrasonic degraded dextran

% IPA	wt. of fractions in g.	% yield	[η]
35	7.4	73.5	0.64
40	1.0	10.1	0.63
45	0.3	3.4	0.56
50	0.1	1.0	0.49
55	not recovered	——	——

Integral and differential distribution curves. The integral distribution curve provides a simple and direct means for comparing the molecular weight or intrinsic viscosity distributions produced by the different procedures of degradation under investigation. Using the dry weights and the intrinsic viscosities of the sub-fractions obtained from fractional precipitation of the degraded polymers resulting from the three different methods, a graph was prepared plotting "Percent Cumulative Weight" precipitated against "Intrinsic Viscosity". Figure 5 represents the resultant integral distribution curves.

In the determination of molecular weight distribution, the differential curve is usually the most revealing. This curve results from plotting the slopes from the integral distribution curves against intrinsic viscosities. These slopes represent the rate of change of cumulative weight with change in the intrinsic viscosity $\frac{dw}{d[\eta]}$. In plotting, slopes were taken from the integral distribution curves at representative intrinsic viscosity intervals and plotted against the intrinsic

viscosities at which they were taken. Figure 6 shows the resultant curves for the different procedures of degradation. In this figure it may be seen that ultrasonic depolyerization results in degraded polymers, most of which lie within a narrow range of molecular weights. In contrast, acid and enzymatic degradation lead to wide ranges of molecular weights, with some evidence for tri- and bi-modal distribution, respectively. Errors in the fractionation procedure and viscosity measurements are not sufficiently large to account for the bi-modal distribution curve for enzyme degraded dextran as an artifact: But there is some possibility that the differential distribution curve for acid hydrolyzed dextran is uni-modal rather than tri-modal, since a smooth curve with only one inflection point may be drawn through the points of the integral distribution of figure 5. The important feature of the distribution obtained for acid-hydrolyzed dextran is the wide range of molecular weights which is represented.

TABLE 8

Results of fractional precipitation of ultrasonic depolymerized dextran B-512 ([η] 0.24–0.26)

Fraction	% IPA	wt. of fraction g	% yield	[η]	Mol. Wt.*
U_1	32.5	27.7	69.3	0.260	108,000
U_2	37.5	4.6	11.5	0.255	———
U_3	42.5	3.1	7.9	0.250	105,000
U_4	47.5	1.5	3.7	0.240	———
U_5	50.0	0.8	2.0	0.190	———

*Obtained by light-scattering method.

TABLE 9

The intrinsic viscosity and molecular weight distribution of various sub-fractions of ultrasonic degraded dextran B-512

Fraction	Sub-fraction	% IPA	% Yield	[η]	Mol. Wt.*
U_1	a	32.5	38.4	0.280	———
	b	35.0	14.6	0.275	118,000
	c	37.5	6.0	0.270	116,000
	d	42.5	1.8	0.230	———
	e	55.0	1.7	0.200	———
U_2	a	37.5	6.3	0.270	114,000
	b	40.0	3.2	0.180	———
	c	55.0	1.0	0.150	———

*Obtained by light-scattering method.

DISCUSSION

The production of degraded dextran for use as a plasma volume expander has been reported by many investigators. Studies on acid hydrolysis on dextran B–512 have been discussed by Wolff *et al.* (1954). Independently, we have obtained similar results. Increase in acid concentration and temperature resulted in a rapid decrease of viscosity.

Factors affecting the ultrasonic depolymerization of dextran B–512 have been explored. It was found that dextran concentration, its molecular weight and the power input of the ultrasonic apparatus are among the main factors that govern the degradation process.

Comparative studies on the molecular weight distribution of degraded dextran B-512 resulting from three methods of depolymerization under investigation showed that partial acid hydrolysis and enzyme degradation through extended incubation (Hamdy, 1953 and Jeanes *et al.*, 1948) led to polymers with a wide range of molecular sizes, while ultrasound produces a degraded dextran with a narrow range of molecular weights.

It would appear from the results that there is an essential difference in the mechanism of these methods of depolymerization. The acid and enzyme degradations involve cleavage of the glycosidic linkage in the molecules to form reducing low molecular weight dextran. In contrast, the cavitation action of ultrasound fractures the molecules to produce non-reducing low molecular weight dextran. This fracturing may involve carbon to carbon bonds with a preferential degradation of higher molecular weight material. The work of Schmid *et al.* (1951) on synthetic polymers supports such a mechanism of degradation. These authors stated that ultrasonic vibrations can break down macromolecules by rupturing covalent bonds. Alexander and Fox (1954) also stated that the breaking of bonds by ultrasound decreased as the molecules became smaller. This mechanism seems to be comparable to what has been reported by Prudhomme and Graber (1949) and Weissler (1950). The cavitation action, which is the important factor in the ultrasonic vibration, has been investigated by Colin (1940), Weissler et al. (1950) and Wise and Ensminger (1952).

The method developed in this work for the fractional precipitation of dextran and for cleanly separating the precipitated dextran gel from the supernatant solution phase by quick freezing of the gel phase was found to be a convenient and rapid procedure. In this method, the higher alcohol content of the solution phase made it possible to freeze the gel phase without freezing the supernatant liquid, and to pour off the supernatant solution without disturbing the gel phase.

SUMMARY

(1) A modified fractionation procedure has been developed for the separation of molecular weight size classes of depolymerized dextran in which dextran is fractionally precipitated from aqueous solution by the addition of isopropyl alcohol and then frozen to convert the gel-like phase of precipitated dextran to a solid, leaving the supernatant solution in liquid form for subsequent separation by decantation.

(2) Factors affecting the degradation of dextran by acid and ultrasound have been investigated.

(3) The molecular weight distributions for the degraded polymers resulting from acid, ultrasound and enzymatic depolymerization were determined. Acid and enzyme degradation resulted in a wide range of molecular sizes, in contrast to ultrasonic degradation which gave a narrow spectrum of molecular weights.

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THE DIGESTIVE ENZYMES OF THE STABLE FLY, *STOMOXYS CALCITRANS* (L.).[1]

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In recent years, an ever-increasing number of publications dealing with the digestive physiology of blood-sucking insect pests have appeared. The greater part of these studies have dealt with digestion in mosquitoes, such as *Aedes aegypti* and *Anopheles quadrimaculatus*, which are known to be vectors of etiological organisms in diseases affecting man.

In this study the stable fly was chosen as a test insect for several reasons, chiefly the relative ease with which large numbers can be reared for experimental purposes. In this insect, both sexes feed upon blood, and do so readily under laboratory conditions. While the stable fly has not been definitely incriminated as a vector of the etiological organism of any human disease, it remains a pest of considerable economic importance due to the vicious bites it inflicts upon man and other animals, particularly livestock.

Rearing and Feeding the Flies. The procedure followed in rearing the insects has already been published (Champlain, *et. al.*, 1954). All blood used for feeding flies was bovine, and contained 250 ml. of 5 percent sodium citrate solution per gallon in order to prevent coagulation.

Flies used in enzyme tests were allowed to feed to repletion upon blood poured over a piece of cotton covering the bottom of a polystyrene plastic dish measuring about 4 in. in diameter and 1 in. in depth. Before it was placed in a cage containing flies, the feeding dish was placed in a pan of warm water until the blood felt tepid to the fingertips. When this was done, flies which had been deprived of both food and water for a 24-hour period began to feed almost immediately.

Experiments indicated that the flies fed most readily when they were about 4 days old. A routine feeding procedure was adopted in which flies were allowed to emerge in a holding cage which was provided with a plastic dish containing cotton soaked with 5 percent sucrose solution as food. At the end of two days, the food dish was removed for a 24-hour period, following which the blood meal mentioned above was placed in the cage.

Equipment and Care of Glassware. In all colorimetric procedures, a Klett-Summerson Model 800–3 photoelectric colorimeter was used. Before readings were made, the machine was adjusted to read zero when a Klett tube containing 5 ml. of distilled water was put in position. A "Magni-Whirl" Model MW1110 water bath operating at $40 \pm 0.5°$ C was used to incubate test mixtures.

For centrifuging, a Sorvall angle centrifuge was used. The rheostat position required to produce a speed of 5500 r.p.m. was determined by means of a stroboscope, and this setting was used exclusively in enzyme runs.

All glassware coming in contact with enzymatic or other proteinaceous material was scrubbed thoroughly with a solution of "*Dreft*" detergent, and then was rinsed several times with tap water. The glassware was then immersed in concentrated nitric acid and allowed to remain there overnight. Three rinses with tap water followed its removal from the acid, and these were followed by four rinses with distilled water. After draining, a rinse of 95 percent ethyl alcohol, followed by

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one of acetone, removed the water. Complete volatilization of the acetone was insured by placing the glassware in an oven operating at 100° C. for about 2 hr.

Qualitative Tests. Since a review of the literature did not disclose any previous work on stable fly digestive enzymes, gross tests were made in order to establish the presence or absence of the various enzymes. If a test was positive, various portions of the alimentary tract, including accessory structures, were tested separately in order to localize the site of secretion. If an enzyme was found to be present in the midgut, but absent in portions of the alimentary tract lying anterior to it, the inference was made that it actually was secreted in the midgut. If the enzyme was present in a structure anterior to the midgut, and also in the midgut, it was inferred that the anterior region was actually the site of secretion and that the enzyme was present in the midgut due to the passage of materials through the alimentary canal. Only one enzyme, a weak salivary amylase, was found in structures anterior to the midgut. All other digestive enzymes encountered in the midgut region appeared to be secreted there.

Due to limitations of space, the results of these qualitative tests will be presented in tabular form (table 3). Detailed descriptions of some of the tests have been omitted, but the type of each test has been indicated. Most of the tests were techniques which have been used for some time and reference to almost any laboratory manual of insect physiology will provide the necessary details. The tests used in proteolytic enzyme experiments, however, are less commonly used, and detailed descriptions of them follow.

Tests for Pepsin-like Enzyme. In the past, tests for pepsin have often been omitted, with a statement that this type of enzyme was not found in insects. However, Greenberg and Paretsky (1955) provide some evidence for the presence of such an enzyme in the house fly. For the sake of completeness, tests were run, using stable flies as an enzyme source.

The substrate, sulfanilic acid-azoalbumin, was prepared according to the method of Tomarelli, Charney, and Harding (1949). Since pepsin-like enzymes are most active in the pH range from about 1.5 to 3.0, separate substrate solutions were made up covering this range by 0.5 pH units. A given solution was prepared by dissolving 250 mg. of azoprotein in about 5 ml. of distilled water. Next, the micro beaker containing the dissolved material was placed on a Beckman Model H–2 pH meter fitted with micro electrodes. The pH of the solution was adjusted to the desired level by dropwise addition of 0.2M HCl, and then the liquid was transferred to a graduated glass cylinder and its final volume was brought to 10 ml. by the addition of a suitable volume of buffer solution of the same pH. This procedure was repeated until individual substrate solutions had been prepared for pH 1.5, 2.0, 2.5, and 3.0. Each solution contained 25 mg. azoprotein/ml.

Chromophoric protein derivatives, such as azoalbumin and azocasein, are formed by the coupling of a diazotized aryl amine with a protein in an alkaline solution. Such azoproteins are completely precipitated by trichloroacetic acid, yielding a colorless filtrate. Digestion of a solution of such proteins by proteolytic enzymes results in the formation of colored fragments which are soluble in trichloroacetic acid (Charney and Tomarelli, 1947). The appearance of color in the trichloroacetic acid filtrate depends upon an intact diazotized amino acid or peptide fragment. The intensity of color in the filtrate following protease activity is a direct function of the proteolytic activity of the enzyme source and its measurement serves as the basis of this technique.

Whole stable fly bodies were used as an enzyme source in order that the enzyme would not be overlooked if it was present somewhere outside of the alimentary tract. The bodies of 16 flies which had been digesting a blood meal for 4 hr. were ground up in a mortar containing a little clean sand and 1 ml. of pH 1.5 buffer solution. The resulting brei was extracted with an additional 3 ml. of buffer solution. The mixture was transferred to a centrifuge tube and centrifuged

at 4500 r.p.m. for 5 min. in order to clear the debris. Aliquots consisting of 0.5 ml. of the centrifuged extract were then placed in each of six micro test tubes, and stored temporarily in the deep freezer. A similar procedure was followed in preparing breis for pH values of 2.0 and 2.5. In the preparation of brei at pH 3.0, double quantities were used and aliquots placed in 12 micro tubes, six of which were boiled 5 min. prior to storage in the deep freezer, and severed as blanks.

When all breis had been prepared, they were removed from the deep freezer and allowed to thaw. Next, 0.5 ml. of buffer solution of the same pH in which the brei had been extracted was added, along with 0.25 ml. of azoalbumin substrate solution at the same pH. All tubes were then placed in the water bath for 4 hr. at 40° C. At the end of this time, all tubes were removed from the water bath and were given what will subsequently be referred to as "the routine protein test". The contents of the micro tubes were poured into numbered centrifuge tubes which contained 1.5 ml. of 10 percent trichloroacetic acid. Each micro tube was then rinsed with an additional 1 ml. of trichloroacetic acid, and this rinse was added to the contents of the appropriate centrifuge tube. The precipitated protein was separated by centrifuging for 10 min. at 5500 r.p.m. Next, 2.5 ml. of the

TABLE 1

Colorimeter readings obtained in protease tests performed on whole bodies of flies

SUBSTRATE	pH	HOURS INCUBATED	LOWEST READING	HIGHEST READING	AVERAGE	CORRECTED AVERAGE
azoalbumin	1.5	4	19	21	19.7	—
"	2.0	4	18	23	20.5	—
"	2.5	4	19	22	20.8	—
"	3.0	4	27	30	28.0	—
"	blank (3.0)	4	28	31	29.3	0
azocasein	7.8	0	30	35	34	6
"	7.8	2	58	65	62	34
"	7.8	4	109	122	115	87
"	7.8	6	166	178	171	143
"	blank (7.8)	6	27	29	28	0

supernatant liquid from each tube were placed in a calibrated Klett colorimeter tube, and then 2.5 ml. of 0.5N sodium hydroxide solution were added to each Klett tube. The addition of the sodium hydroxide solution caused a shift in pH and served to intensify the color. Readings were determined by means of the colorimeter, using the 440 $m\mu$ blue filter.

The amount or the condition of the blood contained in the digestive tracts of the flies had no effect upon the results of the tests since the blood proteins would be precipitated by the trichloroacetic acid, and any colored materials soluble in this acid would be equal in both blanks and runs. The results of these tests are given in table 1. The data show that the readings of all tubes were very low, none exceeding the blank level. Since there was no significant increase in activity in the active tubes, the results were interpreted as being negative for a pepsin-like enzyme.

Tests for Trypsin-like Enzyme. A second chromophoric protein derivative, sulfanilamide-azocasein, was prepared according to the method of Charney and Tomarelli (1947), and their directions were followed in preparing a stock solution. The basis for the technique and the test methods are similar to those used in testing for pepsin, except that pH values in the alkaline range were used.

At the time these tests were made, the pH optimum for the hydrolysis of azocasein by stable fly trypsin was not known, but tests were run using buffer solutions of pH 7.8, since the pH optima of many insect trypsins lie near this value.

The bodies of 44 flies which had been digesting a blood meal for 4 hr. were ground up in a mortar containing a little clean sand and 1 ml. of pH 7.8 buffer solution. The brei was extracted with an additional 10 ml. of buffer solution, and 0.5 ml. aliquots were withdrawn and placed in each of 20 micro tubes. Four of these tubes were boiled for 5 min., serving as blanks. Next, 0.5 ml. of pH 7.8 buffer solution was added to each of the 20 micro tubes, followed by the addition of 0.25 ml. of azocasein stock solution. Four of the active tubes were precipitated immediately by the addition of 1.5 ml. of 10 percent trichloroacetic acid and then tested by means of the routine protein test. The remaining 12 active tubes and the 4 blanks were placed in the water bath operating at 40° C. At the end of 2 hr., 4 hr., and 6 hr., four active tubes were withdrawn and tested; the blanks were tested at the end of 6 hr.

A similar brei was prepared and a similar arrangement of micro tubes was used on the following day, providing 8 replicates.

Results of these tests are given in table 1. The corrected average was obtained by subtracting the average blank reading from the various average readings. These data show a marked increase in optical density as time increases, indicating increasing hydrolysis of the chromophoric protein substrate, and the test was interpreted as being positive for trypsin. A statistical analysis of the data, using

TABLE 2

Synopsis of qualitative enzyme tests

ENZYME	TEST USED	RESULT	SITE OF SECRETION
Invertase	Benedict test for reducing sugars.	Positive	Midgut
Lactase	Phenylhydrazine test for reducing sugars.	Negative	—
Lipase	Change in pH of alkaline substrate-brei mixture. Bromthymol blue indicator used.	Positive	Midgut
Amylase	Non-appearance of blue color in starch substrate when tested with iodine indicator.	Positive (weak)	Salivary glands
Pepsin-like proteinase	Hydrolysis of azoalbumin substrate.	Negative	—
Trypsin-like proteinase	Hydrolysis of azocasein substrate.	Positive	Midgut

a t-test of a regression coeffiicient, showed that the rate of change differed signficantly from zero at a 2 percent confidence level. The calculated "t" value was 9.889.

Similar tests run upon separate portions of the gut produced substantially the same results when the midgut was tested.

In testing for lactase activity, the results were interpreted as being negative even though precipitates formed in all tubes, including blanks and controls, when they were given the osazone test for reducing sugars. These precipitates were identified as glucosazone and lactosazone. Precipitates also formed when phenylhydrazine reagent was added to aliquots of midgut brei to which no substrate had been added. This phenomenon may have been due to the normal glucose level of the blood ingested by the flies. Final interpretation was based upon the fact that microscopic examination of the osazone precipitates failed to reveal crystals of galactosazone, a product which should have appeared if the reducing sugars had been end-products of lactase activity. Table 2 is a synopsis of qualitative tests.

QUANTITATIVE PROTEASE TESTS AND RESULTS

pH Optima for Stable Fly Trypsin. Earlier authors (Baldwin, 1949; Northrop, 1939) have pointed out that an optimum pH is characteristic of a given enzyme, though under certain special conditions and in certain groups of enzymes the pH optimum may vary. This is true of the proteolytic enzymes, different optima being found with different protein substrates. Therefore, the next group of tests were run in order to determine the pH optimum of fly trypsin in the hydrolysis of azocasein and azoalbumin, respectively.

pH Optimum, Tryptic Hydrolysis of Azoalbumin. Since trypsin-like proteinases generally are most active at alkaline pH values between 7 and 10, preliminary experiments, using azoalbumin as a substrate, were run covering this pH range.

Individual substrate solutions were made up for each pH value tested by dissolving 250 mg. of azoalbumin in 5 ml. of distilled water, and then bringing the pH of the solution to the desired point by dropwise addition of 0.2M sodium hydroxide solution, followed by dilution to a final volume of 10 ml. by the addition of an appropriate volume of buffer solution of that pH. This procedure produced solutions containing 25 mg. azoprotein/ml.

TABLE 3

Colorimeter readings obtained in determination of pH optimum

SUBSTRATE	pH	LOWEST READING	HIGHEST READING	AVERAGE OF 8	CORRECTED AVERAGE
azoalbumin	7.7	198	200	199.5	176.5
"	7.8	206	209	208	185
"	7.9	213	215	214.5	191.5
"	8.0	208	210	209	186
"	8.1	200	201	200.5	177.5
"	blank (8.1)	22	24	23	0
azocasein	7.6	248	251	250	227
"	7.7	251	253	252	229
"	7.8	256	258	256.8	233.8
"	7.9	248	250	249	226
"	8.0	245	249	248	225
"	blank (8.0)	22	25	23	0

Results of these tests indicated a maximum of activity occurring at about pH 8. Therefore, the range lying between pH 7 and 9 was tested, being covered by 0.2 pH units. These tests verified the results obtained in the first runs, almost equal values being obtained at pH 7.8 and 8.0.

Final tests were made at pH values 7.7, 7.8, 7.9, 8.0, and 8.1. The preparation of the brei was altered somewhat in order to minimize random error due to variations in separate breis by following a procedure which would produce quite uniform aliquots. The midguts of 60 flies which had been digesting a blood meal for 4 hr. were dissected out in small groups which were stored in the deep freezer until all had been accumulated. Following thawing, they were ground up and transferred to a small graduated glass cylinder. The final volume was brought to 3 ml. by the addition of an appropriate volume of distilled water. Use of aliquots of 0.1 ml. provided enzymatic material equivalent to that contained in 2 midguts.

Aliquots of brei were placed in each of 24 micro tubes. These tubes were arranged in groups of four each, each group representing a pH value. pH 8.1 was represented by eight tubes. Next, 0.9 ml. of buffer solution of the desired pH was placed in the corresponding tubes. At this point, four of the pH 8.1 tubes were boiled for 5 min., serving as blanks. Then 0.25 ml. of azoalbumin substrate

solution of the appropriate pH was placed in each corresponding micro tube. All tubes were incubated in the water bath at 40° C. for 4 hr. At the end of this time, they were removed and given the routine protein test. The runs were repeated, with a total of eight replicates, the results of which are given in table 3 and figure 1.

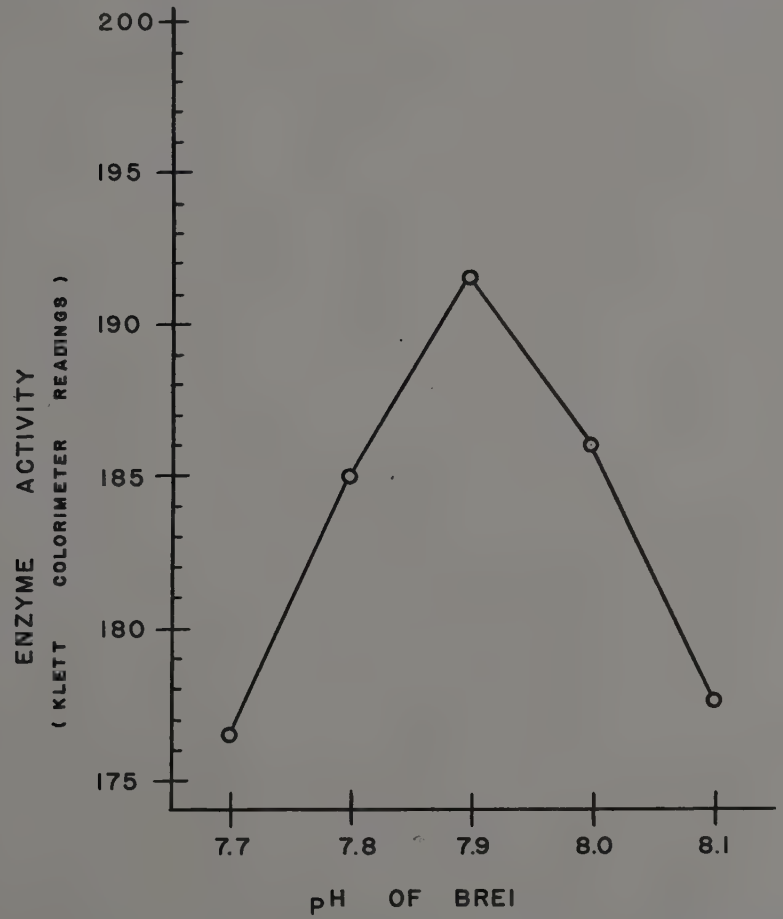

FIGURE 1. pH optimum of stable fly protease with azoalbumin as substrate. *Brei:* midguts of adult flies, both sexes, 4 hr. after a blood meal. *Incubation:* 4 hr. at 40° C at pH values shown.

These results show a maximum of activity occurring at pH 7.9, indicating that this value represents the optimum pH for the hydrolysis of azoalbumin by stable fly trypsin under the conditions of the experiment.

pH Optimum, Tryptic Hydrolysis of Azocasein. The procedure used in these tests was identical to that just described for determining the pH optimum for hydrolysis of azoalbumin.

Preliminary experiments indicated a maximum of activity at about pH 7.8. Therefore, final runs were made using the pH values 7.6, 7.7, 7.8, 7.9, and 8.0. Blanks were run at pH 8.0, and the routine protein test was employed. Eight replicates were used. Results are given in table 3 and figure 2.

These data show a maximum of activity occurring at pH 7.8, indicating that this value represents the optimum pH for the hydrolysis of azocasein by stable fly trypsin under the conditions of the experiment.

Protease Activity Related to Time. The phenomenon of an increase in enzymatic activity following feeding has been known for over half a century, Biedermann (1898) being one of the earliest writers to mention its occurrence. Fisk and Shambaugh (1952) note such an increase in female *Aedes* mosquitoes. They also note an initial drop in protease activity immediately following feeding, a change which was not detected by Fisk (1950). Fisk and Shambaugh (1952) also

TABLE 4

Colorimeter readings obtained in testing relationship of Protease activity to time of digestion of a blood meal

TIME OF DIGESTION	LOWEST READING	HIGHEST READING	AVERAGE	CORRECTED AVERAGE
0	39	39	39	19
5 min.	39	41	40	20
15 min.	50	53	52	32
30 min.	60	62	61	41
1 hr.	86	91	89	69
2 hr.	102	107	105.5	85.5
3 hr.	177	183	180.5	160.5
6 hr.	283	292	287.5	267.5
9 hr.	335	341	339.2	319.2
12 hr.	355	366	360.5	340.5
13 hr.	386	392	388	368
14 hr.	383	391	386	366
15 hr.	363	369	365.2	345.2
18 hr.	330	336	334	314
24 hr.	303	310	306	286
blank	19	21	20	0

demonstrated that protease activity in the female mosquito, *Aedes aegypti*, is at its maximum about 18 hr. after a blood meal, after which time the protease activity begins to diminish. The subsequent experiments were designed in order to determine how much time intervened between feeding and maximum proteolytic activity in the stable fly.

Preliminary tests indicated a maximum of enzyme activity at about 12–14 hr. after feeding.

Flies fed upon citrated bovine blood were captured in an aspirator, and groups of 20 were allowed to digest the meal for the time periods given in the table of results. When this period had elapsed, they were killed and the midguts were dissected out. Since a large number of flies was used, and since different groups represented different digestion intervals, dissection was performed by group, and the breis were held frozen at −15° C until all had been prepared. Twenty fly guts were used in each brei, diluted to a final volume of 1 ml. (Use of 0.1 ml. aliquots provided the previously-adopted standard of 2 midguts per aliquot.) Azocasein stock solution at pH 7.8 and buffer solution of the same value were used.

Aliquots of 0.1 ml. of each brei were placed in each of two micro tubes. Nine-tenths ml. of pH 7.8 buffer solution was added to each tube. At this point, two tubes were boiled for 5 min., serving as blanks. (Four zero-hour tubes were set

up, two being boiled and two being retained as active). Then 0.25 ml. of azocasein stock solution was added to each micro tube, and all tubes incubated in the water bath at 40° C for 4 hr. At the end of this time, they were given the routine protein test. Similar tests, run on four different days, provided 8 replicates. Results are given in table 4 and figure 3.

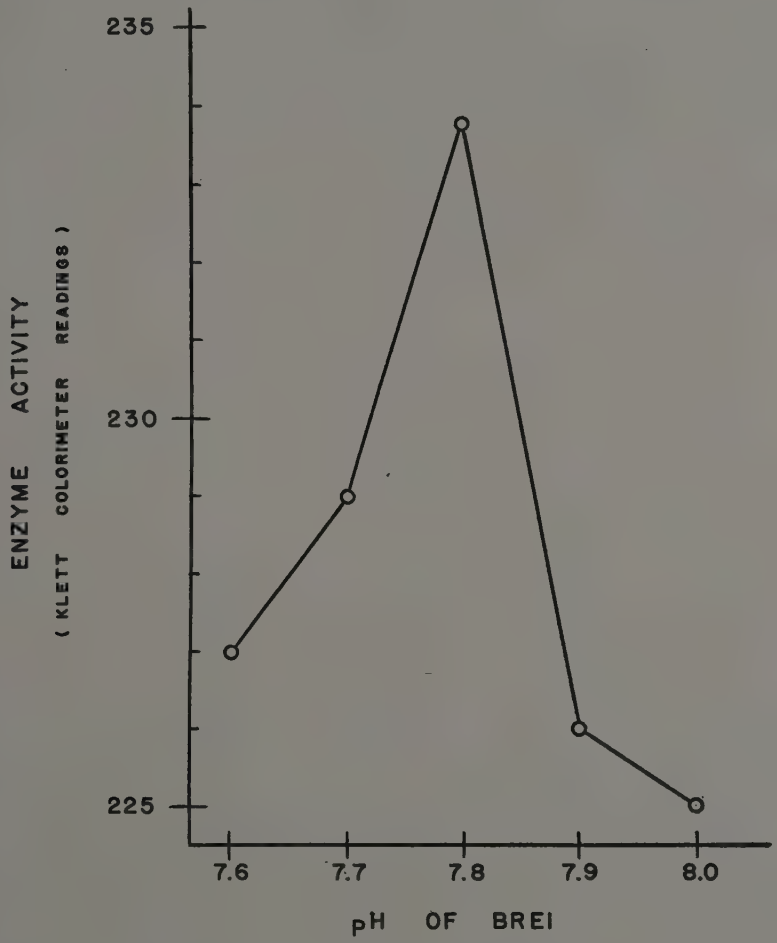

FIGURE 2. pH optimum of stable fly protease with azocasein as substrate. *Brei:* midguts of adult flies, both sexes, 4 hr. after a blood meal. *Incubation:* 4 hr. at 40° C at pH values shown.

These results showed a sharp rise in activity after the first 5 min. This rise continued for about 6 hr., at which time the rate decreased somewhat. Maximum proteolytic activity appeared to occur about 13 hr. after a blood meal. The activity began to decline after the maximum was reached and continued to drop during the remainder of the time interval covered in the test.

As noted above, Fisk and Shambaugh (1952), working with *Aedes aegypti* females, observed a significant decrease in proteolytic activity in the first 5 min. following a blood meal. The results of the tests under discussion do not show a similar phenomenon in the stable fly. The readings obtained at 5 min. are approximately the same as those obtained at zero hours. The last-named authors attribute their lowered 5-minute readings to a possible "depletion" of the enzyme by its substrate. It is worth noting, however, that a comparison is being made between two insects in which ingested blood is stored in quite different ways. It has been known for a long time that when a mosquito ingests blood, this liquid passes directly to the stomach, while other fluids, such as nectar, go first to the crop and the dorsal diverticula. In the muscoid bloodsuckers, such as *Glossina* and *Stomoxys*, ingested blood passes into the crop, where no digestion or denaturation occurs. The crop functions simply as a storage organ, and quantities of blood are released from it periodically, this blood entering the midgut.

This divergence being the case, variation in phenomena related to blood feeding logically might be expected. Phenomena related to feeding upon nectar or sucrose solution should be in more general agreement, because the relative mechanisms are more similar.

TABLE 5

Colorimeter readings obtained in tests for protease activity in sucrose-fed stable flies

TIME OF DIGESTION	LOWEST READING	HIGHEST READING	AVERAGE	CORRECTED AVERAGE
Unfed	42	45	43	21
5 min.	42	45	43.2	21.2
30 min.	44	46	45	23
1 hr.	54	58	56.2	34.2
2 hr.	49	51	50	28
4 hr.	43	46	45.7	23.7
8 hr.	45	47	46.2	24.2
16 hr.	45	47	46	24
24 hr.	42	45	44.3	22.3
blank	21	23	22	0

The proteolytic enzymes tested are localized in the midgut in both the mosquito and the stable fly. Currently-accepted theories of the formation of an enzyme-substrate complex imply a rather intimate combination between molecules of the enzyme and the substrate, and in the case of the mosquito, the sudden admission of a large volume of blood substrate directly into the region where the enzymes are localized conceivably could produce the "depletion effect". On the other hand, the mechanism in the stable fly regulating admission of blood to the midgut is such that only a comparatively small part of the total volume of ingested blood would reach the midgut in the initial 5-minute period. This relatively small amount of substrate might not be sufficient to produce a noticeable "depletion effect", especially in view of the fact that enzyme secretion might increase even in this short time, to the point where it would counterbalance any small initial depletion. In any event, no significant drop in proteolytic activity 5 min. after a blood meal was observed in these experiments on the stable fly.

The experimental establishment of a maximum of protease activity at about 13 hr. apparently would contradict the statement of Metcalf and Flint (1951) that "a single stable fly feeds several times a day, taking a drop or two of blood each time". On the basis of observed results, it would seem that two feedings per day would be a maximum. At the end of 24 hr., a very small amount of blood,

which is dark in color and almost rubbery in consistency, remains in the gut. Under laboratory conditions, absolute digestion of a blood meal would require a little more than 24 hr.

It should be noted that the statement of Metcalf and Flint, mentioned above, pertains to stable flies living under natural conditions, while the observations made here pertain to flies living under laboratory conditions. Wild flies undoubtedly are more active than flies in laboratory cages, and probably digestion proceeds more rapidly in the former. In addition, Fisk (1950) observed that the physical nature of the ingested blood may be a factor in digestive efficiency. All blood used in the laboratory was treated with sodium citrate to prevent coagulation, and this may slow down the overall digestive rate, especially if coagulation is a normal digestive phenomenon, which would appear to be the case in the stable fly, as

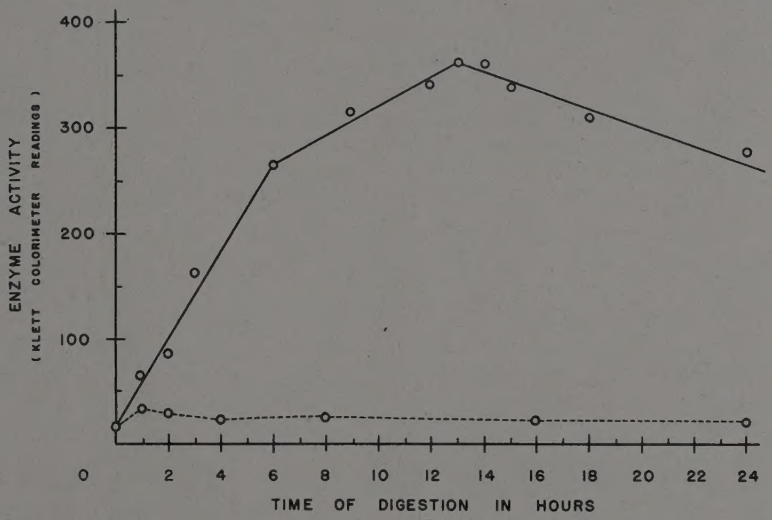

FIGURE 3. Protease activity at various intervals after feeding. (Solid line—blood-fed series; broken line—sucrose-fed series.) *Brei:* midguts of adult flies, both sexes. *Substrate:* azocasein. *Incubation:* 4 hr. at 40° C and pH 7.8.

indicated by the rubbery consistency of the blood remaining in the gut at the end of 24 hr. Some insects secrete salivary anti-coagulins which would tend to neutralize the discrepancy just mentioned, but according to Cornwall and Patton (1914), such a salivary anti-coagulin is absent in *Stomoxys*.

In view of the different environments occupied by wild flies and laboratory flies, as well as the differing foods they ingest, the statement of Metcalf and Flint may not be incorrect.

Protease Activity as Related to Food. It was originally planned that a similar series of tests would be run using flies which had fed upon skimmed milk. All attempts to obtain predictable feeding responses failed completely when this material was offered as food.

Since the ingestion of food stimulates the production of all enzymes normally found, rather than one specific enzyme, tests were included to measure the effect of the ingestion of a non-proteinaceous food, sucrose, on protease activity.

Flies were allowed to digest a meal of 5 percent sucrose solution for various intervals as set forth in table 5. Breis were made up and tested on three different

days as described in the preceding section relating to time, providing six replicates. Results are given in table 5 and figure 3.

These data show a slight initial increase in protease activity during the first hour, after which the activity declines and remains close to the value obtained using unfed flies. The general pattern of activity is similar to that obtained by Fisk and Shambaugh (1952), although they cite 30 min. as the time at which peak activity occurred. They do not present data for 1-hour and 2-hour insects. According to table 5, maximim protease activity occurs in the midgut of the stable fly about 1 hr. after a meal of sucrose solution.

SUMMARY

Qualitative tests were positive for trypsin, invertase, lipase, and amylase. Tests for pepsin and lactase were negative.

A pH optimum for the hydrolysis of sulfanilic acid-azoalbumin by stable fly trypsin was determined as being pH 7.9.

A pH optimum for the hydrolysis of sulfanilamide-azocasein by stable fly trypsin was determined as being pH 7.8.

When protease activity was related to time of digestion of a blood meal, maximum activity was obtained at about 13 hr.

Protease activity following a meal of sucrose solution showed a slight increase for about an hour, but this activity quickly declined and remained close to the residual value.

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NOTICE

On October 15, 1949 our present subscription prices became effective. Since that date the number of pages printed per volume has been increased more than 50%. Our costs have risen more than 75%. For these reasons we find it necessary to increase annual subscription prices of The Ohio Journal of Science to **$6.00** postpaid (U.S.A.) and **$6.50** postpaid (Outside U.S.A.). These prices become effective with subscription to Volume 57 (1957).

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The Joint Administrative Board
of the Ohio Journal of Science
November 11, 1955

PROGRESS REPORT III. THE OHIO FLORA PROJECT

The Ohio Flora Project is a long-range research program aiming to assemble sound scientific data as to what plants grow where in Ohio. Current efforts are concentrated on the native and naturalized vascular plants. This work is a project of the Ohio Academy of Science and is being directed by a committee which was appointed in 1950 by the president of the Academy. The committee is happy to report continued progress.

General procedure is to collect records of known herbarium specimens of Ohio plants. From these records mimeographed preliminary lists are being prepared in which distribution by counties is reported. (It should be noted that most of these records must still be verified.) On the basis of these preliminary lists such specimens as are needed to fill in the gaps are being collected. As is feasible, published papers dealing with portions of the Ohio Flora will appear.

The major tangible evidences of progress to date are enumerated and briefly described below:

1. In the spring of 1953 there was distributed a mimeographed preliminary list of the woody plants of Ohio. Since that time, much work has been done on keys, text, and preliminary distribution maps.

2. A manuscript dealing with the genus *Crataegus* of Ohio is now being prepared.

3. A manuscript dealing with the genus *Salix* is also being prepared for publication.

4. In the spring of 1954 a preliminary list of Ohio grasses was distributed to those interested.

5. In the spring of 1955 a preliminary list of Ohio pteridophytes was mimeographed and mailed to many of you.

6. A similar bulletin dealing with Ohio legumes was sent out during the summer of 1955.

7. The latest major step has been to request that records of Ohio Compositae be submitted. A preliminary list of this group will be forthcoming.

8. The fact that hundreds of specimens have been collected in an effort to add new county records indicated that the preliminary mimeographed lists are significant. A record, of course, means that a specimen is in an herbarium.

Such are the highlights of progress. Those interested in further details and willing to contribute even a few botanical specimens may add their names to the ever growing mailing list on request to the secretary.

This is the third of a series of short notes dealing with this project which have appeared in this Journal. The previous notes were published as follows: (1) 52: 34, Jan. 1952, and (2) 55: 64, Jan. 1955.

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